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Alternative Herbicide Control Options for Glyphosate-Resistant Palmer Amaranth (Amaranthus Palmeri)

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ALTERNATIVE HERBICIDE CONTROL OPTIONS FOR GLYPHOSATE-RESISTANT PALMER AMARANTH (*AMARANTHUS PALMERI*)

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ABSTRACT

The occurrence of glyphosate-resistant (GR) Palmer amaranth has prompted a shift in weed management strategies worldwide. Studies were conducted with the aim to (1) establish and compare the degree of tolerance of GR Palmer amaranth populations; (2) assess the efficacy of glufosinate, tembotrione, 2,4-D or dicamba, applied alone or tank-mixed, on Palmer amaranth with higher tolerance to glufosinate in the greenhouse and corn field, and (3) establish the mechanism involved in the tolerance of Palmer amaranth to glufosinate. Tembotrione, 2,4-D, dicamba, and glufosinate applied at 1x controlled 80 to 100%, 98 to 100%, 84 to 100%, and 94 to 100% Palmer amaranth, respectively. Differential response of Palmer amaranth populations to the test herbicides existed. The potential of selecting for resistance was highest in tembotrione, followed by dicamba. In the tank mixture test, all herbicides applied individually at 1x rate controlled Pra-C population 99 to 100% in the greenhouse and 91 to 100% in the field study. In corn, the control in Pra-C, Mis-C, and STF-C populations was 33 to 54% for tembotrione, 68 to 89% for 2,4-D, and 96 to 100% for glufosinate applied at their commercial rates. The study showed that half rates of 2,4-D and glufosinate can be applied, only in combination, without significantly compromising Palmer amaranth control. The majority of glufosinate + tembotrione and some glufosinate + dicamba mixtures were not compatible; glufosinate + 2,4-D mixtures were generally additive and in few cases, synergistic. The reduced efficacy from antagonism was overcome by mixing 1x rates of the herbicides. Pra-C (tolerant) had 2-folds higher tolerance than Lee-A (susceptible), with LD_{50} values of 344 and 141 g ha^{-1}, respectively. The basal activity of the tolerant population was 20% higher than that of the susceptible. Tolerance to glufosinate is certainly due to higher baseline activity of GS in the tolerant plants, which would require more herbicide molecule to cause substantial inhibition.

This thesis is approved for recommendation

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CHAPTER I

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats., subgenus *Acnida*, subsection *Saueranthus*) is one of the most common weedy species in field crops in the southern United States (US). The genus *Amaranthus* has 75 species worldwide (Pratt and Clark 2001; Steckel 2007) and approximately 15 species are found in Arkansas (Hunter 2001). Based on their growth habit and flowering, Steckel (2007) grouped the weedy species of *Amaranthus* into three groups. Group one consists of tall, upright, dioecious (male and female flowers on separate plants) pigweeds. This group includes common waterhemp (*Amaranthus rudis* Sauer), Palmer amaranth, and tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer]. These species are very competitive with crops because of their upright and branching growth habit. The second group of *Amaranthus* consists of prostrate growing pigweeds that are monoecious (the plant has both male and female flowers). This group includes spiny amaranth (*Amaranthus spinosus* L.), tumble pigweed (*Amaranthus albus* L.), and prostrate pigweed (*Amaranthus blitoides* S. Wats.). Tumble and prostrate pigweeds are commonly found around the edges of fields and pastures. The last group is composed of tall, upright pigweeds that are also monoecious. This group includes redroot pigweed (*Amaranthus retroflexus* L.), smooth pigweed (*Amaranthus hybridus* L.), and green amaranth (*Amaranthus viridis* L.).

Within the first group of *Amaranthus*, Palmer amaranth is the most competitive species. Palmer amaranth can have as high as 130% more biomass at 4 weeks after planting than redroot pigweed, smooth pigweed, common waterhemp, spiny amranth, and tumble pigweed (Sellers et al. 2003). Palmer amaranth has wider leaves than those of its relatives like waterhemp (Mallard 2009), which gives it a comparative better light interception advantage its counterparts. The fast growth habit (up to 1.5 cm d^{-1}) and season-long emergence (Jha and Norsworthy 2005; Keeley et al. 1987) gives farmers a narrow window to control Palmer amaranth. Palmer amaranth plants,

therefore, have a higher likelihood of escaping late topical-applied herbicide. Palmer amaranth also grows vigorously (up to 2 m tall in a growing season); because of this, the growing plants dominate competition with crops for light, water, nutrients, and space. Palmer amaranth is also a prolific seed producer, with estimates of over 600,000 seeds per plant (Sellers et al. 2003); this enables it to develop and replenish seed banks tremendously. Palmer amaranth is a C4 plant (Downtown 1975) and therefore more efficient in water and light use. Palmer amaranth's photosynthetic capacity is relatively even higher than other C4 plants. Palmer amaranth out-competes most other plant species for light because its leaves are able to solar-track thereby remaining perpendicular to the direct solar rays (Ehleringer 1983). Palmer amaranth is also drought tolerant thereby enabling it to survive and grow under moisture stress conditions in which most plants would hardly thrive in. Physiologically, Palmer amaranth roots have been reported to penetrate compact soils and access nitrogen better and faster than soybean (Place et al. 2008). These and other characteristics make Palmer amaranth historically a difficult weed to control especially in cotton production. Morgan et al. (2001) reported reduced cotton canopy volume by 35 and 45% in the presence of 1 and 10 plants $9.1m^{-2}$, respectively, at 6 and 10 weeks after cotton emergence. The same densities above reduced cotton lint yield by 13 and 54%. Apart from lowering crop yields, the presence of Palmer amaranth in a crop field can increase harvesting time by 2 to 3.5-fold in dry land cotton (Smith et al. 2000).

The use of pesticides has a long history in the US and elsewhere in the world. This dates back to as early as soon after World War II (Hodgson 1991). According to the chemical usage data for 2009, herbicides constituted 95% of all pesticides being used on weight basis in the US (NASS 2009). Pesticide use increased by 700 million kg between 1996 and 2008, mainly because of increased hectarage resulting from adoption of herbicide-tolerant crops. Since the

commercialization of glyphosate-resistant (GR) crops in mid-1990s, growers have used glyphosate more than any other herbicide to manage weeds. To date, glyphosate continues to provide an effective and economically viable management tool in non-resistant weed species, including Palmer amaranth. The rapid adoption of GR soybean (*Glycine max* L.), maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), canola (*Brassica napus* L. and *B. rapa* L.), alfalfa (*Medicago sativa* L.), and sugarbeet (*Beta vulgaris* L.) production is largely because of the economic advantage, simplicity, and superior weed control that the GR technology delivers to growers (Green 2009; Duke and Powles 2009). In addition, the GR technology is more environmentally friendly than the weed management technologies that it replaced. This makes glyphosate a herbicide with combined characteristics that no other herbicide has provided so far. The approximate area under transgenic GR cultivars worldwide is 70 million ha, the largest proportion of which (45 million ha), is in the US (Price et al. 2011).

The unprecedented extensive use of glyphosate alone over space and time exerted intense selection pressure, consequently, GR weed biotypes including Palmer amaranth have evolved (Culpepper et al. 2006; Heap 2012). The world's first GR Palmer amaranth was confirmed in Georgia in 2005. Soon after this confirmation, studies on Palmer amaranth populations suspected of being resistant to glyphosate intensified in North Carolina, South Carolina, Tennessee, and Arkansas and this led to confirmation of GR Palmer amaranth in these states. Currently, the presence of GR Palmer amaranth has been confirmed in 14 states (Alabama, Arkansas, Georgia, Missouri, North Carolina, South Carolina, Tennessee, Mississippi, Florida, Illinois, Michigan, Louisiana, New Mexico, and Virginia) in the US (Culpepper et al. 2006; Heap 2012; Nichols et al. 2009; Norsworthy et al. 2008; Steckel et al. 2008;). In Arkansas, GR Palmer amaranth was first confirmed in Mississippi County, in 2006. Palmer amaranth is also resistant to

dinitroanilines (Gossett et al. 1992), photosystem II (PSII) inhibitors (Heap 2012), and acetolactate synthase (ALS) inhibitors (Horak and Peterson 1995; Sprague et al. 1997). Glyphosate-resistant common waterhemp, another amaranth species, has also been confirmed (Legleiter and Bradley 2008). This narrows down further the available control options for resistant biotypes of this troublesome weed. Some of the reasons being put forward by scientists for the evolution of resistance in weeds include over-dependence on a single herbicide, relying on a single mode of action year in and out, and sequential applications of the same herbicides within the cropping cycle (Bond et al. 2010). Presence of resistant Palmer amaranth to glycines, ALS, PSII and dinitroanilines has affected farming practices and weed management strategies in upland crops. Although new cultivars with resistance to glyphosate and 2,4-D or dicamba or other combinations of traits are being developed, practicing proper weed management remain critical; otherwise, growers will, in the near future, be faced with another serious weed resistance problem if the lessons learned from managing glyphosate resistance are ignored (Price et al. 2011). Herbicides with modes of action other than glycines, ALS or PSII must therefore be integrated into weed management programs in order to effectively control Palmer amaranth populations resistant to the above-mentioned herbicides.

Herbicide-resistant weeds are populations that have evolved the ability to survive the commercial rates of a herbicide that previously controlled them (WSSA 1998). Resistance to herbicides may occur naturally due to selection or may be induced thereby leading to a genetic mutation in the plant, which is passed on to future generations (Prather et al. 2000). If the herbicide becomes a selection pressure, susceptible weeds will be killed whereas resistant ones will survive, reproduce, and become dominant if application of the same herbicide continues. A

weed that has become resistant due to a genetic shift is known as a resistant biotype. These resistant biotypes are most often visually indistinguishable from their susceptible counterparts.

There are 20 herbicide-resistant biotypes in Arkansas. Some examples are goosegrass (*Eleusine indica* L.) with resistance to trifluralin (first reported in 1989); Palmer amaranth with resistance to ALS inhibitors, barnyardgrass (*Echinochloa crus-galli* L.), with resistance to synthetic auxins (like quinclorac), horseweed (*Conyza canadensis* L.*),* and common ragweed with resistance to glycines (Smith et al. 2000). It is estimated that there are about 6,000 sites and over 250,000 ha infested with herbicide-resistant weeds in Arkansas and they infest mainly cotton, rice (*Oryza sativa* L.), soybean, and wheat (*Triticum aestivum* L.) fields. Palmer amaranth, present in an estimated area of between 40,000 and 400,000 ha, primarily in cotton and soybean, has the most widespread resistant biotypes in Arkansas (Heap 2012). Rice flatsedge (*Cyperus iria* L.), with resistance to glyphosate (Heap 2012), is the most recently discovered resistant biotype in Arkansas. Some potential herbicides for managing GR weeds like Palmer amaranth are glufosinate, 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba and tembotrione. The advancements in herbicide resistant crops, especially 2,4-D and dicamba will have an impact on weed management strategies in the southern and many other parts of the US.

Even with the most highly active herbicides, it is not always possible to have an economical and effective weed control from a single herbicide. Tank mixing of herbicides is generally aimed at enhancing herbicides efficacy. However, there is potential for interaction among the herbicides, which compromise their activities by reducing or increasing the efficacy of individual herbicides in the mixture. There are three possible interactions of tank-mixed herbicides. The resulting interaction between two or more herbicides can be additive, synergistic, or antagonistic. In the first case, the control achieved by individual herbicides is equal to that

achieved by tank-mixed application. In situations where the sum of the activities from all herbicides in the mixture is greater or lower than when the herbicides are applied separately, the herbicides are said to be synergistic or antagonistic, respectively (Hatzios and Penner 1985). Antagonism measures or defines the interaction between herbicides and not the agronomic usefulness of tank mixing. Therefore, an antagonistic mixture could sometimes still be useful for managing resistance in weeds.

The goal of this study was to investigate the tolerance of glyphosate-resistant Palmer amaranth populations to glufosinate, tembotrione, 2,4-D, and dicamba. Cotton and soybean resistant to HPPD inhibitor, 2,4-D, or/and dicamba herbicides are being developed for commercialization (Green and Owen 2011; Herman et al. 2005; Stuebler et al. 2008). It is important that the response of Palmer amaranth to foliar-applied herbicides, in particular, glufosinate, dicamba, tembotrione, and 2,4-D continue to be investigated. Therefore, the objectives of this research were to (1) establish and compare the degree of tolerance of GR Palmer amaranth populations; (2) assess the efficacy of glufosinate, tembotrione, 2,4-D or dicamba, applied alone or tank-mixed, on the control of Palmer amaranth with higher tolerance to glufosinate in the greenhouse and corn field, and (3) establish the mechanism involved in the tolerance of Palmer amaranth to glufosinate.

Literature Cited

Bond, J., T. Eubank, K. Smith, and B. Scott. 2010. Glyphosate-resistant Palmer amaranth and ryegrass management. Mississipi Agricultural and Forest Extension Station information sheet 1351. University of Arkansas.

Burgos, N. R., E. A. L. Alcober, A. Lawton-Rauh, B. Rauh, L. Estorninos Jr., T. M. Tseng, and K. L. Smith. 2009. The spread and population genetics of glyphosate-resistant Palmer amaranth in Arkansas. Pages 94-104 in D. M. Oosterhuis, ed. Summaries of Arkansas Cotton Research. University of Arkansas, Fayetteville, AR.

Culpepper A. S., A. C. York, S. M. Brown, W. W. Hanna, J. W. Davis, W. K. Vencill, T. L. Grey, T. M. Webster, and J. M. Kichler. 2006. Glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) confirmed in Georgia. Weed Sci. 54:620-626.

Culpepper, A. S., T. M. Webster, L. M. Sosnoskie, and A. C. York. 2010. Glyphosate-resistant Palmer amaranth in the United States. Pages 195-212 in V. J. Nandula, ed. Glyphosate resistance in crops and weeds: History, development and management. New York: John Wiley and Sons Inc.

Duke, S. O. and S. B. Powles. 2009. Glyphosate-resistant crops and weeds: Now and in the future. AgBioForum 12:346-357.

Ehleringer J. 1983. Ecophysiology of *Amaranthus Palmeri*, a sonoran desert summer annual. Oecologia 57:107-112.

Gossett, B.J., E.C. Murdock, and J.E. Toler. 1992. Resistance of amaranth (*Amaranthus palmeri*) to dinitroaniline herbicides. Weed Technol. 6:587-591.

Green, J. M. 2009. Evolution of glyphosate-resistant crop technology. Weed Sci. 57:108-117.

Green, J. M. and M. D. K. Owen. 2011. Herbicide-Resistant Crops: Utilities and limitations for herbicide-resistant weed management. J. Agric. Food Chem. 59:5819-5829.

Hatzios, K. K. and D. Penner. 1985. Interactions of herbicides with other agrochemicals in higher plants. Rev. Weed Sci. 1:1-63.

Heap, I. M. 2012. Glycine resistant weeds by country and species. http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go. Accessed: March 29, 2012.

Herman, P. L., M. Behrens, S. Chakraborty, B. M. Chrastil, J. Barycki, and D. P. Weeks. 2005. A three-component dicamba O-demethylase from *Pseudomonas maltophilia*, strain DI6: gene isolation, characterization, and heterozygous expression. J. Biol. Chem. 280:24759-24767.

Hodgson, E. 1991. Pesticides: past, present and future. Pages 3-12 in E. Hodgson, R. M. Roe, N. Motoyama, eds. Pesticides and the future: toxicological studies of risks and benefits. North Carolina State University, Raleigh, NC.

Horak, M. J. and D. E. Peterson. 1995. Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol. 9:192-195.

Hunter, C. G. 2001. Wild flowers of Arkansas. 6th ed. Little Rock, Ar: The Ozark Society Foundations. 14 p.

Jha, P. and J. K. Norsworthy. 2005. Palmer amaranth emergence timing: An aid to develop integrated weed management strategies. Clemson University Research Forum.

Keeley, P. E., C. H. Carter, and R. J. Thullen. 1987. Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci. 35:199-204.

Legleiter, T. R. and K. W. Bradley. 2008. Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci. 56:582-587.

Mallard, D. J. 2009. Management of Glyphosate Resistant Palmer Amaranth in Liberty Link® Soybeans. MSc. Thesis. Martin, TN. University of Tennesse. 4-5 p.

Morgan, G. D., J. M. Chandler, and P. A. Baumann. 2001. Competitive impact of Palmer amaranth (*Amaranthus Palmeri*) on cotton (*Gossypium hirsutum*) development and yield. Weed Technol. 15:408-412.

Nichols, R. L., J. Bond, A. S. Culpepper, et al. 2009. Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) spreads in the Southern United States. Resist. Pest Manag. News. 18:8-10.

Norsworthy, J. K., G. M. Griffith, R. C. Scott, K. L. Smith, and L. R. Oliver. 2008. Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) in Arkansas. Weed Technol. 22:108-113.

Place, G., D. Bowman & M. Burton, and T. Rufty. 2008. Root penetration through a high bulk density soil layer differential response of a crop and weed species. Plant Soil. 307:179-190.

Prather, T. S., J. M. Ditomaso, and J. S. Hold. 2000. Herbicides resistance definition and management strategies. Division of Agriculture and Natural Resources. The Regents of University of California.

Price, A. J., K. S. Balkcom, S. A. Culpepper, J. A. Kelton, R. L. Nichols, and H. Schomberg. 2011. Glyphosate-resistant Palmer amaranth: A threat to conservation tillage. J. Soil Water Conserv. 66:265-275.

Sellers, B. A., R. J. Smeda, W. G. Johnson, J. A. Kendig, and M. R. Ellersieck. 2003. Comparative growth of six *Amaranthus* species in Missouri. Weed Sci. 51:329-333

Simth, D. T., R. V. Baker, G. L. Steele. 2000. Palmer amaranth (*Amaranthus palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). Weed Technol. 14:122-126.

Smith, D. T., G. L. Steele, and R. V. Baker. 2000. Palmer amaranth (*Amaranthus Palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). Weed Technol. 14:122-126.

Sprague, C. L., E. W. Stoller, L. M. Wax, and M. J. Horak. 1997 Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) resistance to selected ALS-inhibiting herbicides. Weed Sci. 45:192-197.

Steckel, L. E. 2007. The dioecious *Amaranthus* spp.: Here to stay. Weed Technol. 21:567-570.

Stuebler, H., H. Kraehmer, M. Hess, A. Schulz, C. Rosinger, C. 2008. Global changes in crop production and impact trends in weed management-an industry view. Page 309-319 in Proceedings of the 5th annual meeting. Vancouver, BC: Int. Weed Sci. Cong.

USDA-NASS (U.S. Department of Agriculture–National Agricultural Statistics Service). 2009. Agricultural Chemical Use. Washington, D.C USDA National Agricultural Statistics Service. http://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/. Accessed July 16 2012.

WSSA. 1998. Resistance and tolerance definitions. Weed technol. 12:789.

CHAPTER II

Literature Review

Palmer Amaranth Interference in Crops. Palmer amaranth (*Amaranthus palmeri* S. Wats.) is a major weed problem in the southern Great Plains. The problem of Palmer amaranth has increased throughout the southern Great Plains more than all the other weedy *Amaranthus* species, causing concern among farmers and researchers (Currie et al. 1998; Horak 1997). The occurrence of populations resistant to herbicides that inhibit acetolactate synthase, photosynthesis at photosystem II, and 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) (Culpepper et al. 2006; Gaeddert et al. 1997; Heap 2012; Horak and Peterson 1995; Norsworthy et al. 2008; Sprague et al. 1997; Whitaker et al. 2007) limits further the herbicide options for Palmer amaranth control. The competitive ability of Palmer amaranth is due to its fast growth (Horak and Loughin 2000), high fecundity (Sellers et al. 2003), good light interception, and high water use efficiency (Ehleringer 1983). Palmer amaranth is a C4 plant (Dowton 1975; Kellogg 1999) and is therefore generally more efficient in utilizing resources than most crops that are C3. Regardless of having similar water use efficiencies, Palmer amaranth survives water stress better than other C4 species like corn (Massinga et al. 2001; Wiese and Vandiver 1970). Palmer amaranth can, therefore, grow and reproduce under conditions in which most crops would not thrive in. The ability to outcross with other dioecious species like *Amaranthus rudis* Sauer (Franssen et al. 2001; Tranel et al. 2002; Wetzel et al. 1999), and season-long emergence (Jha 2008; Keeley et al. 1987) are other attributes that have made Palmer amaranth a successful weed.

Palmer amaranth has been reported to reduce yield or interfere with harvesting mostly in soybean (*Glycine max* L.) and cotton (*Gossypium hirsutum* L.). At densities of 0.32 and 10 plants m^{-1} of row, Palmer amaranth reduced cotton and soybean yields by 28 and 68%, respectively (Klingaman and Oliver 1994; Smith et al. 2000). Yield loss has also been reported in grain sorghum (*Sorghum bicolor* L.), sweet potatoes [*Ipomoea batatas* (L.) Lam], and corn. Each

additional Palmer amaranth plant per 15 m of row decreased grain sorghum yield by 97, 190, and 92 kg ha^{-1} at three sites in Oklahoma (Moore et al. 2004). Competition studies conducted in North Carolina showed that the presence of 0.5 to 6.5 Palmer amaranth plants m^{-1} in sweet potatoes can reduce yield by 56 to 94% (Meyers et al. 2010). Massinga and Currie (2002) reported that 0.5 to 8 Palmer amaranth plants m^{-1} of row resulted into 11 to 91% corn yield reduction in Kansas. Rule (2006) reported a 39 and 52% yield loss in irrigated corn with 1 and 4 Palmer amaranth plants m^{-1}. Liphadzi and Dille (2006) predicted a 13% yield loss from three Palmer amaranth plants that escaped herbicide treatment m^{-1} of row. They further reported dryland and irrigated corn yield losses of 6 to 60% and 5 to 38%, respectively, for Palmer amaranth densities of 0.25 to 6 plants m^{-1}. The competitive ability of Palmer amaranth with crops is mainly dependent on emergence timing of the weed. Delayed emergence of the weed favors rapid crop establishment and early development. However, these late emerging Palmer amaranth plants can escape herbicide sprays and eventually contribute to seed bank replenishment (Hartzler et al. 2004; Nordby and Hartzler 2004).

Weed Control Programs in Major Crops of the South. The south, which comprises the Southeast, Delta states, Appalachians, and Southern plains produce several crops ranging from extensively cultivated monocultures of cotton, soybean, corn, rice, tobacco, sugarcane, and peanuts to intensively grown vegetables and citrus fruits (USDA 2011). Cotton, soybeans, and corn are the most commonly produced crops, with production in almost every state in the southern US. Prior to introduction of glyphosate-resistant cultivars, weeds in conventional cultivars were controlled with herbicides applied as burndown or before planting of the crop. For instance, until pyrithiobac (2-chloro-6-[4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid) became available in 1996, no herbicide was available for overtop application in cotton (Webster et al.

2000). In soybean, a herbicide program typically utilized a PRE for grass control followed by a two-pass of ALS-inhibiting POST and this provided great weed control except for large-seeded weeds like common cocklebur (*Xanthium strumarium* L.), morningglory species (*Ipomoea* spp), sicklepod (*Senna obtusifolia* L.). Weeds with high rates of reproduction, such as *Amaranthus* spp. were also difficult to control (Price et al. 2011). Tillage was also advocated for managing weeds because it can reduce weed biomass between rows (Ali et al. 2011; Snipes and Muller 1992) and control bieannial weeds (Brown and Whitwell 1988). However, the inability to incorporate herbicides or implement inter-row cultivation reduced control options. The introduction of transgenic technology has enabled growers to reduce tillage while achieving season-long weed control (Wilcut et al. 1995). Currently, growers have chemical weed option with several herbicides that can be applied prior to or after crop emergence, including glyphosate, glufosinate, and tembotrione (Table 1). More cultivars with resistance traits to other herbicides like dicamba and 2,4-D are being developed.

Evolution of Herbicide Resistance. Following the introduction of GR technology in 1996, adoption of GR crops in the United States has been rapid especially in cotton, soybean, and has further increased in recent years due to introduction of GR maize (Dill et al. 2008). The occurrence of ALS-, ACCase-, and PSII-resistant weeds reduced the number of herbicide active ingredients that can be used for weed control. Glyphosate provided good control for ALS-, PSII-, ACCase-resistant biotypes. The unprecedented rise in glyphosate use has partially supported the decline in use of other herbicides and subsequent evolution of GR weeds (Conner et al. 2003; Firbank et al. 2000; Powles 2003; Shaner 2000; Watikinson et al. 2000; Young 2006). The evolution of resistance to glyphosate has been more rapid than anticipated (Bayliss 2000). However, the changes that have taken place within the weed communities are not surprising

considering the level of selection pressure that GR technology imparted on the agroecosystem (Owen 2008). Currently, there are several weeds that are resistant to glyphosate (Table 1). Several resistance mechanisms to glyphosate are involved in weeds, the most common being reduced cellular transport to active meristematic tissues and insensitive altered EPSPS (Powles and Preston 2006). In the family *Amaranthus*, the resistance mechanisms that have been reported include enzyme insensitivity in ALS resistance (Burgos et al. 2001; Sprague et al. 1997), point mutation to ALS- and PSII-inhibiting herbicides (Hirschberg et al. 1987; Tranel and Wright 2002; Wise et al. 2009) and gene amplification in glyphosate resistance (Gaines et al. 2009).

Table 1. Weeds species of major crops of the World that have evolved resistance to glyphosate[1].

Weed species	Crop situation	Year confirmed	Reference[2]
spiny amaranth (*Amaranthus spinosus*)	GR cotton	2012	Heap 2012*
johnsongrass (*Sorghum halepense*)	Soybean	2005	Vila-Aiub et al. 2007
Palmer amaranth (*Amaranthus palmeri*)	GR cotton	2005	Culpepper et al. 2006
Asiatica dayflower (*Commelina diffusa*)**	Cotton, peanut, soybean	2005	Owen and Zelaya 2005
common lambsquartes (*Chenopodium album*)**	GR soybean	2005	Owen and Zelaya 2005
tropical spiderwort (*Commelina benghalensis*)**	GR cotton	2004	Culpepper et al. 2004
velvetleaf (*Abutilon theophrasti*) **	GR soybean	2005	Owen and Zelaya 2005
buckhorn plantain (*Plantago lanceolata*)	Orchard and vineyard	2005	Heap 2005
common ragweed (*Ambrosia artemisiifolia*)	GR soybean	2004	Heap 2005
common waterhemp (*Amaranthus tuberculatus*)	GR soybean	2005	Owen and Zelaya 2005
horseweed (*Conyza canadensis*)	GR cotton, soybean	2000	VanGessel 2001
Italian ryegrass (*Lolium perenne* ssp. *multiflorum*)	Orchard	2001	Perez and Kogan 2003
rigid ryegrass (*Lolium rigidum*)	Summer and winter crops	1996	Powles et al. 1998

[1] Table adapted from Nandula et al. (2005).

[2] A representative peer-reviewed publication is shown in case of multiple reports of evolved resistance in same weed species.

* “Based on anecdotal evidence. Resistance not confirmed by peer reviewed publications”, (Nandula et al. 2005).

** Weed species with inherently more resistance to glyphosate.

Management Strategies for Herbicide-Resistant Palmer Amaranth. Due to the occurrence of resistant biotypes to ALS-, EPSPS-, and PSII-inhibiting herbicides in different parts of the US, Palmer amaranth control options are specific to a particular region. However, the choice of herbicides to use excludes the families to which resistance is documented or being suspected. Generally, GR Palmer amaranth control requires an integration of different management strategies including crop rotation, use of herbicides with different modes of action (Diggles et al. 2003; Neve et al. 2011) applied sequentially or in tank mixture (Clewis et al. 2008; Everman et al. 2009; Scroggs et al. 2007). The general herbicide recommendations for GR Palmer amaranth management in cotton, corn, and soybean include a burndown program, followed by preemergence (PRE) or preplant incorporated (PPI) and postemergence (POST) treatments. POST herbicides are applied at ≤8 cm stage of Palmer amaranth, otherwise control is significantly reduced as the plants grow taller. Some of the recommended herbicides for management of GR Palmer amaranth are paraquat and flumioxazin (burndown); *S*-metolachlor, diuron, fomesafen, pendimethalin, and trifluralin (PPI or PRE); and glufosinate, 2,4-dichlorophenoxyacetic acid (2,4-D), tembotrione, and dicamba as POST herbicides (Table 2). Glufosinate, 2,4-D, tembotrione, and dicamba were selected for the studies reported herein.

Table 2. Management options and application timings for Palmer amaranth control in major crops of the southern US.

	Herbicide option[a]			
Crop	PRE or PPI[b]	POST[c]	Lay-by[d]	Reference
Cotton	fomesafen	glyphosate	prometryn	Neve et al. 2011
	diuron	fluometuron[e]	glyphosate (directed)	
		S-metolachlor[e]	glufosinate	
		glufosinate	flumioxazin	
			MSMA	
Corn		glyphosate		Neve et al. 2011
		S-metolachlor[e]		
		atrazine		
		glufosinate		
Soybean	flumioxazin	fomesafen		Scott and Smith 2010
	S-metolachlor	glyphosate		
	trifluralin	glufosinate		
	pendimethalin	acetochlor[e]		

[a] Herbicides are applied individually or tank mixed depending on the need.

[b,] Applied at 1 month before planting; [c] applied at planting in corn and cotton or at 2, 4, and 6 wk after planting in cotton; [d] applied at 8 wk after planting in cotton.

[e] PRE herbicide applied at second POST to provide control weeds emerging in the late season.

Tank Mixing of Herbicides for Palmer Amaranth Control. Even with the most highly active herbicides, it is not always sustainable to have an economical and effective weed control from a single herbicide. Tank mixing of herbicides, whether applied PPI, PRE, or POST, is mainly aimed at enhancing the overall chemical control and expanding the weed control spectrum (Wilcut et al. 1995). Another advantage of tank mixing over single-product application is to have a single-pass spray of herbicides that would otherwise need multiple passes. This saves time and is cost-effective. Tank mixtures are usually recommended on the basis of increasing weed control. However, there is potential for interaction among the herbicides, which compromises the activities of individual herbicides, thereby reducing or increasing the efficacy or crop injury (Damalas 2004).

Both increased and reduced activities of tank mixtures involving glufosinate, 2,4-D, dicamba, or mesotrione with other herbicides have been reported. Chafin et al. (2010) reported that glufosinate (0.47 kg ha^{-1}), 2,4-D (0.56, 0.84 or, 1.2 kg ha^{-1}), or dicamba (0.28, 0.56 or 1.2 kg ha^{-1}) applied alone controlled GR Palmer amaranth less than 80%. Glufosinate activity increased on GR Palmer amaranth and pitted morningglory by 25 to 32% when tank mixed with dicamba or 2,4-D. Palmer amaranth and morningglory control was 91 and 100% when glufosinate was tank mixed with 2,4-D or dicamba, respectively. It was further observed in the same study that the efficacy of glufosinate on broadleaf signalgrass was reduced by 8% when mixed with any dose of 2,4-D or dicamba. Glufosinate applied alone controlled carpetweed (*Mollugo verticillata* L.) 99% and the level of control was not affected by addition of dicamba or 2,4-D. On the other hand, dicamba and 2,4-D applied alone controlled 60 and 66% carpetweed, respectively. Chuah et al. (2008) evaluated the response of goosegrass to reduced rates of ametryn plus glufosinate or glyphosate tank-mixes. He reported that five of the nine combinations were synergistic whereas

four were antagonistic in greenhouse experiments. Antagonism was overcome by mixing higher rates of ametryn (0.08 kg ha^{-1}) and glufosinate (0.05 kg ha^{-1}), and this tank mix controlled goosegrass 50% higher than when glufosinate was applied alone. Antagonism has also been reported in glufosinate-atrazine tank mix for horseweed control in rye (*Secale cereale* L.) (Wilson et al. 1985). Dicamba antagonism of nicosulfuron activity on yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes] is commonly reported in North Dakota (Zollinger et al. 2001).

Glufosinate. Glufosinate [monoammonium 2-amino-4-(hydroxymethylphosphinyl) butanoate] inhibits the activity of glutamine synthetase (GS) enzyme, which plays a pivotal role in nitrogen (N) metabolism by catalyzing the condensation of glutamate and ammonia (NH_3) to form glutamine (Cox 1996). The GS enzyme inhibition by glufosinate in plants is manifested by NH_3 accumulation, inhibition of amino acid synthesis, inhibition of photosynthesis, and severe damage to plant tissues, ultimately, resulting into plant death. Glufosinate is a broad-spectrum, POST contact herbicide with limited mobility in the plant (Droege and Puehler 1992; Droege-Laser et al. 1994). It is used as a preplant burndown treatment or over the top of LibertyLink® crops. The availability of glufosinate-tolerant cotton, soybeans, and corn varieties provides growers an alternative herbicide for controlling Palmer amaranth that is resistant to other herbicides.

Researchers have previously conducted studies on Palmer amaranth control with glufosinate. An experiment conducted in Macon County, Georgia, showed that glufosinate applied alone or tank mixed with 2,4-D or dicamba controlled 73 and 91% of the GR Palmer amaranth in cotton (Chafin et al. 2010). Glufosinate applied alone controlled pitted morningglory (*Ipomoea lacunosa* L.) 97%; 100% control was achieved when applied in combination with 2,4-

D or dicamba. Chafin et al. (2010) further observed that although glufosinate was more effective than glyphosate in controlling glyphosate-resistant Palmer amaranth, the application timing mostly influenced glufosinate efficacy. Palmer amaranth plants taller than 7 cm were not adequately controlled by glufosinate. Drake et al. (2009) reported 90 to 100% control by 0.45 kg ai ha^{-1} glufosinate when applied within 3 weeks after emergence (WAE) or on 10- to 15-cm tall Palmer amaranth. In an experiment conducted by Doherty et al. (2009), 0.59 and 0.82 kg ha^{-1} glufosinate controlled 100% of 8- and 15-cm tall Palmer amaranth and >90% control of 22-, 30-, 61-, and 71-cm Palmer amaranth. The researchers further reported that all glufosinate treatments suppressed seed production of Palmer amaranth.

Differential tolerance of weed species to glufosinate exists and has been attributed to application rate, plant species (Steckel et al. 1997), application timing (age of the plant at herbicide application) (Sellers et al. 2004), humidity, temperature (Anderson et al. 1993; Coetzer et al. 2001; Kumaratilake and Preston 2005; Petersen and Hurle 2001), and absorption and translocation (Mersey et al. 1990). Ridley and McNally (1985) observed that the differential tolerance of weed species to glufosinate was not due to differences in the degree of GS inhibition, but was mostly because of differences in absorption, translocation, and metabolism of the herbicide. Contrary to what was reported by Ridley and McNally (1985), studies by Haas and Muller (1987), Jansen et al. (2000), and Neto et al. (2000) did not associate metabolism with differential response of weed species to glufosinate. However, 2-acetamido-4-methylbutanoic acid (N-acetyl-glufosinate; NAG) was found to be the main metabolite (Ruhland et al. 2004) in glufosinate-resistant plants. The fatty acid metabolites 3-(hydroxymethylphosphinyl)propionic acid (MPP) and 2-hydroxy-4-(hydroxymethylphosphinyl)butanoic acid (MHB) have also been identified to be stable compounds in non-transgenic plants expressing low acetylation activity

(Droge-Laser et al. 1994; Muller et al. 2001). Glufosinate-resistant Palmer amaranth has not been documented (Heap 2012). However, resistance to glufosinate has been reported in goosegrass (*Eleusine indica* L) (Chuah et al. 2010; Jalaludin et al. 2010) and in Italian ryegrass (*Lolium perenne* L.) (Avila-Garcia and Mallory-Smith 2011).

Tembotrione. Tembotrione {2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-(trifluoroethoxy)methyl]benzoyl]-1,3-cyclohexanedione} belongs to the hydroxyphenylpyruvate-dioxygenase (HPPD) inhibiting herbicides. HPPD is a key enzyme in the synthesis of plastoquinones and tocopherols. Plastoquinones are essential cofactors for enzymes such as phytoene desaturase, which aid in biosynthesis of carotenoids. Tocopherols (vitamin E) are antioxidants that promote growth and endow tolerance to stress (Freigang et al. 2008). The depletion of carotenoids in the chloroplasts results into vulnerability of the plant to damage by excessive light and eventually the plant dies (Freigang et al. 2008; Hess 2000). Tembotrione was discovered in 1997 and launched as a commercial herbicide 10 years later (2007/2008) in Austria, Hungary, USA, and Brazil (Almeida Dan et al. 2012). The absorption rate of tembotrione by treated plants foliage is very high and fast probably because tembotrione translocates within the plant both symplastically (phloem) and apoplastically (xylem). After an uptake period of 6 h, between 68 and 79% of the applied ^{14}C-tembotrione was determined in jimsonweed (*Datura stramonium* L.) and alexandergrass [*Brachiaria plantaginea* (Link) A. S. Hitchc.], with 56 and 39% of the total translocated to the rest of the plants within 48 h, respectively (Schulte and Köcher 2009). Small amounts of the herbicide can still enter the plant via the roots if in contact with the treated soil. Tembotrione is labeled for POST use in corn in the US. The inclusion of a safener isoxadifen-ethyl provides higher selectivity to regular field corn and popcorn (Waddington and Young 2006).

The efficacy of HPPD inhibiting herbicides (tembotrione, mesotrione, and topramezone) applied alone or mixed with atrazine or/and other herbicides has been evaluated on some weed species. Mesotrione has been post-applied for the control of broadleaf weeds such as common lambsquarters (*Chenopodium album* L.), *Amaranthus* species, jimsonweed (*Datura stramonium* L.), spurred anoda (*Anoda cristata* L.), velvetleaf (*Abutilon theophrasti* Medik.), wild radish (*Raphanus raphanistrum* L.), wild mustard (*Sinapis arvensis* L), henbit (*Lamium amplexicaule* L.), and carpetweed (Armel 2002). Bollman et al. (2008) reported that giant foxtail (*Setaria faberi* Herrm.) control was greater with tembotrione or topramezone than with mesotrione alone or mixed with atrazine. In the same experiment, common lambsquarters, velvetleaf, and common ragweed (*Ambrosia artemisiifolia* L.) were controlled 98% or greater with the HPPD-inhibiting herbicides when mixed with atrazine. In an experiment aimed at evaluating HPPD and their tank-mix combinations for weed control in corn, tembotrione applied at 0.092 kg ha^{-1} controlled Palmer amaranth, pitted morningglory, prickly sida (*Sida spinosa* L.), broadleaf signalgrass (*Urochloa platyphylla* (Nash) R.D. Webster), and velvetleaf between 90 and 100%. Tembotrione controlled 87% of entireleaf morningglory (*Ipomoea hederacea* var. *integriuscula*). The addition of atrazine increased the efficacy of tembotrione (Bararpour et al. 2011). Not much has been documented on the efficacy of tembotrione on Palmer amaranth.

Until recently, HPPD-inhibiting herbicides were among the two remaining classes of herbicides (the other being glutamine synthetase inhibitor) with no confirmed herbicide-resistant weeds (Green and Owen 2011; Heap 2011). However, recent studies reveal that tall waterhemp (*Amaranthus tuberculatus* Sauer) in Illinois is resistant to several POST applied HPPD inhibiting herbicides (Hausman et al. 2011); resistance to glufosinate has been reported in goosegrass (*Eleusine indica* L) (Chuah et al. 2010; Jalaludin et al. 2010) and in Italian ryegrass (*Lolium*

perenne L.) (Avila-Garcia and Mallory-Smith 2011). The close genetic relatedness and spatial proximity of tall waterhemp to Palmer amaranth may potentially result in the resistant-gene flow through cross-pollination thereby resulting in the evolution of HPPD-resistant Palmer amaranth populations. Likewise, selection pressure on Palmer amaranth populations by HPPD inhibitors can also result in the evolution of HPPD-resistant Palmer populations.

2,4-dichlorophenoxyacetic acid. 2,4-dichlorophenoxyacetic acid (2,4-D) was developed during World War II and became famous as a component of Agent Orange that was used during the Vietnam War (Tu et al. 2001). To date, 2,4-D continues to be one of the most commonly used herbicides. A variety of 2,4-D products such as Weedar 64, Formula 40, Butoxone, and Butyrac are commercially available on the market. According to Mithila et al. (2011), the mechanism of action of auxinic herbicides like 2,4-D in sensitive dicots involves binding of the herbicide to the receptor protein and active transportation via a common carrier protein into plant cells where it stimulates increased synthesis of abscisic acid (ABA) and ethylene. Although not well understood, it is also speculated that 2,4-D might be acidifying the plants' cell walls (Tu et al. 2001). Low rates of 2,4-D are also believed to stimulate protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), which cause uncontrolled cell division and elongation, consequently resulting in the destruction of plant cells. 2,4-D is a selective herbicide developed for the control of broadleaf weeds and is probably the oldest herbicide. 2,4-D has provided an effective and economical postemergence control of a variety of broadleaf weeds in crops and non-croplands.

The use of 2,4-D for Palmer amaranth control is not a new strategy. In as early as 1945, 2,4-D provided unique control to Palmer amaranth, dandelion (*Taraxacum officinale*), and *Oxalis* weeds and was regarded as the principal herbicide for broadleaf weed control in turf grass (Davis

1945). Today, 2,4-D is customarily applied in combination with other herbicides such as glyphosate, dicamba, paraquat to expand the weed control spectrum. In an experiment aimed at determining the effectiveness of late-season herbicide applications for control and seed suppression of glyphosate-resistant Palmer amaranth in Arkansas, Jha et al. (2009) found that 2,4-D provided the greatest control when compared with glufosinate, dicamba, or pyrithiobac. 2,4-D applied at 1.07 kg ai ha^{-1} controlled 66% GR Palmer amaranth biotypes from Mississippi and Lincoln counties, AR. Nandula et al. (2011) reported that 2,4-D controlled >90% of GR tall waterhemp from Mississippi soybean fields. 2,4-D is usually effective on small Palmer amaranth plants (about 5-to 7-cm tall), but the herbicide has low residual activity, which allows a new cohort of emerging Palmer amaranth to get established, in the absence of supplemental control measures.

The first case of weed resistance to 2,4-D was reported in Hawaii, USA, in 1957 on spreading dayflower (*Commelina diffusa* Burm. F) (Hilton 1957). Over the past six decades of existence, 29 weed species across 16 plant families have evolved resistance to 2,4-D, worldwide (Heap 2012). Palmer amaranth resistance to 2,4-D has not been reported. However, tall waterhemp, another *Amaranthus* species has been found to be resistant to 2,4-D in southeast Nebraska (Bernards et al. 2012). This resistant biotype was reported to be 10-fold more tolerant to 2,4-D than the susceptible biotype in 2009, and had plants surviving 64 times the commercial rate of 2,4-D (1.12 kg ha^{-1}) in 2010.

Dicamba. Dicamba (2-methoxy-3,6-dichlorobenzoic acid) is another selective herbicide registered for the postemergence control of certain broadleaf weeds and woody plants. It was registered in the US in 1967 and is widely used in agricultural, industrial, and residential settings (US EPA 2006). According to the EPA Pesticide Sales and Usage report for 2000/2001, dicamba

was the seventh most commonly used conventional pesticide in the home and garden market sector and ranked 24th in a list of the most commonly used agricultural herbicides (Kiely et al. 2004). Dicamba is an auxin agonist and it triggers excessive production of auxins that cause rapid division of the plant cells thereby resulting into uncontrolled growth of the stems, petioles, and leaves of sensitive plants. This results into epinasty and destruction of the vascular tissues. Weed control is achieved in 5 to 7 d.

Dicamba has been evaluated in Palmer amaranth control. Jha et al. (2009) reported that dicamba caused 31% mortality to two GR Palmer amaranth biotypes from Mississippi and Lincoln counties, AR, when applied at 0.56 kg ai ha^{-1}. Doherty et al. (2009) also reported that dicamba applied at 0.28 and 0.56 kg ai ha^{-1} controlled 100% of the 7-cm Palmer amaranth. However, the same rates controlled 65% of 23-and 30-cm Palmer amaranth, but it did suppress seed production. It was further observed that late-season applications of dicamba, glufosinate, or 2,4-D did not suppress seed production to the extent that could prevent replenishment of the soil seed bank and future spread of the GR population. Auxinic or growth regulator herbicides such as 2,4-D and dicamba may have multiple sites of action and are therefore less likely to select for resistance (Warwick 1991). Although development of dicamba-resistant crops like soybeans and cotton (Herman et al. 2005; Stuebler et al. 2008; Green and Owen 2011) will provide an additional option for weed control, the risk of selecting for resistance in Palmer amaranth, Kochia (*Kochia scoparia* L.), horseweed (*Conyza canadensis* L.), and common waterhemp is estimated to be high (Crespo 2011). So far, no resistance to dicamba has been documented in *Amaranthus*.

Literature Cited

Ali, K., S. K. Khalil, Z. Hussain, F. Munsif, I. U. Din, M. Waqas, and Wagma. 2011. Effect of various tillage methods and nitrogen management on weeds and maize performance. Wee Sci. Res. 17:253-256.

Anderson, D. M., C. J. Swanton, J. C. Hall, and B. G. Mersey. 1993. The influence of soil moisture, simulated rainfall, and time of application on the efficacy of glufosinate-ammonium. Weed Res. 33:149-160.

Armel, G. R. 2002. Weed management in conventional, no-till, and transgenic corn with mesotrione combinations and other herbicides. Ph. D. dissertation. Blacksburg, VA. Virginia Polytechnic Institute and State Univ. 155 p.

Avila-Garcia, W. V. and C. Mallory-Smith. 2011. Glyphosate-resistant Italian ryegrass (*Lolium perenne*) populations also exhibit resistance to glufosinate. Weed Sci. 59:305-309.

Bararpour, M. T., L. R. Oliver, and C. G. Bell. 2011. Comparison of HPPD inhibitors for weed control programs in corn. Page 16 in Proceedings of the 64th annual meeting. San Juan, PR: Southern Weed Science Society.

Bayliss, A. D. 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest Manag. Sci. 56:299-308.

Bernards, M. L., R. J. Crespo, G. R. Kruger, R. E. Gaussoin, and P. J. Tranel. 2012. A waterhemp (*Amaranthus tuberculatus*) population resistant to 2,4-D. Weed Sci. 60:379-384.

Bollman, J. D., C. M. Boerboom, R. L. Becker, and V. A. Fritz. 2008. Efficacy and tolerance to HPPD-inhibiting herbicides in sweet corn. Weed Technol. 22:666-674.

Brown, S. M. and T. Whitewell. 1988. Influence of tillage on horseweed (*Conyza canadensis*). Weed technol. 2:269-270.

Burgos, N. R., Y. Kuk, and R. E. Talbert. 2001. *Amaranthus palmeri* resistance and differential tolerance of *Amaranthus palmeri* and *Amaranthus hybridus* to ALS inhibitor herbicides. Pest Manag. Sci. 57:449-457.

Chafin, J. E., A. S. Culpepper, and L. B. Braxton. 2010. Palmer amaranth, benghal dayflower, carpetweed, pitted morningglory, and broadleaf signalgrass response to glufosinate applied alone or mixed with 2,4-D or dicamba. Beltwide Cotton Conferences, New Orleans, Louisiana.

Christopher, J. T., S. B. Powles, and J. A. M. Holtum. 1992. Resistance to acetolactate synthase-inhibiting herbicides in annual ryegrass (*Lolium rigidum*) involves at least two mechanisms. Plant Physiol. 100:1909-1913.

Chuah, T. S., B. J. Nor Asmah, T. S. Cha, Sayed M. Z. Hasan, and I. B. Sahid. 2008. The Use of reduced rates of herbicide combinations in tank-mixes for goosegrass (*Eleusine indica* (L.) Gaertn.) control. World Appl. Sci. 3:358-362.

Coetzer, E., K. Al-Khatib, and T. M. Laughin. 2001. Glufosinate efficacy, absorption, and translocation in amaranth as affected by relative humidity and temperature. Weed Sci. 49:8-13.

Conner, A. J., T. R. Glare, and J. P. Nap. 2003. The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment. Plant J. 33:19-46.

Cox, C. 1996. Herbicide factsheet: Glufosinate. J. Pest. Reform. 16:15-19.

Crespo, R. J. 2011. Herbicide-Resistant Risk Assessment: Response of common Nebraska weeds to dicamba dose. MSc. thesis. Lincoln, NE. University of Nebraska 52-55p.

Cruz-Hipolito, H., M. D. Osuna, A. Heredia, J. P. Ruiz-Santaella, and R. De Prado. 2009. Nontarget mechanims involved in glyphosate tolerance found in *Canavalia ensiformis* Plants. J. Agri. Food Chem. 57:4844-4848.

Culpepper A. S., A. C. York, S. M. Brown, W. W. Hanna, J. W. Davis, W. K. Vencill, T. L. Grey, T. M. Webster, and J. M. Kichler. 2006. Glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) confirmed in Georgia. Weed Sci. 54:620-626.

Damalas, C. A. 2004. Herbicide Tank Mixtures: Common Interactions. Int. J. Agric. Biol. 6:209-212.

Davis, F. F. 1945. What's been observed in the tests of 2,4-D on weeds. Golfdom 19:11-17.

De Almeida Dan, H., A. L. De Lemos Barroso, R. S. De Oliviera Junior, L. G. De Moraes Dan, S. De Oliveira Procopio, and J. Constantin. 2012. Potential use of tembotrione (HPPD-inhibitor herbicide) in grain sorghum. Pages 171-184 in Andrew J. Price, ed. Weed Control. Croatia. Intech Publishers.

Dickson, J. W., R. C. Scott, N. R. Burgos, R. A. Salas, and K. L. Smith. 2011. Confirmation of glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) in Arkansas. Weed Technol. 25:674-679.

Doherty, R. C., K. L. Smith, J. A. Bullington, and J. R. Meier. 2009. Palmer amaranth control with dicamba and glufosinate as influenced by weed size and herbicide rate. Pages 105-107 in D. M. Oosterhuis eds. Summaries of Arkansas Cotton Research. University of Arkansas, Fayetteville, AR.

Downton, W. J. 1975. The occurrence of C4 photosynthesis among plants. Photosynthetica 9:96-105.

Drake, D. M., L. R. Oliver, and M. T. Barapour. 2009. Palmer amaranth (*Amaranthus Palmeri*) control in soybean. Page 13 in Proceedings of the 13th research conference. Fayetteville, AR: Arkansas Crop Protection Association.

Droege, W., I. Broer, and A. Puehler. 1992. Transgenic plants containing the phosphinothricin-Nacetyltransferase gene metabolize the herbicide L-phosphinothricin (glufosinate) differently from untransformed plants. Planta 187:142-151.

Droege-Laser, W, U. Siemeling, A. Puehler, and I. Broer. 1994. The metabolites of the herbicide LPhosphinothricin (Glufosinate) - Identification, stability and mobility in transgenic, herbicide-resistant, and untransformed plants. Plant Physiol. 105:159-166.

Ehleringer J. 1983. Ecophysiology of *Amaranthus Palmeri*, a sonoran desert summer annual. Oecologia 57:107-112.

Firbank, L.G. and F. Forcella. 2000. Genetically modified crops and farmland diversity. Science 289:1481–1482.

Franssen, A. S., D. Z. Skinner, K. Al-Khatib, M. J. Horak, and P. A. Kulakow. 2001. Interspecific hybridization and gene flow of ALS resistance in Amaranthus species. Weed Sci. 49:598-606.

Freigang, J., B. Laber, G. Lange, and A. Schultz. 2008. The biochemistry of pyrasulfotole. http://www.bayercropscience.com/BCSWeb/CropProtection.nsf/id/EN_3rdArticle012000/$file/03_Freigang.pdf. Accessed: March 6, 2012.

Gaeddert, J. W., D. E. Peterson, and M. J. Horak. 1997. Control and cross-resistance of an acetolactate synthase inhibitor-resistant Palmer amaranth (*Amaranthus palmeri*) biotype. Weed Technol. 11:132-137.

Gaines, T. A., W. Zhang, D. Wang, B. Bukun, S. T. Chisholm, D. L. Shaner, S. J. Nissen, W. L. Patzoldt, P. J. Tranel, A. S. Culpepper, T. L. Grey, T. M. Webster, W. K. Vencill, R. D. Sammons, J. Jiang, C. Preston, J. E. Leach, and Philip Westra. 2009. Gene amplification confers resistance in Amaranthus palmeri. Proc. Nat. Acad. Sci. 107:1029-1034.

Gossett, B. J., E. C. Murdock, and J. E. Toler. 1992. Resistance of amaranth (*Amaranthus palmeri*) to dinitroaniline herbicides. Weed Technol. 6:587-591.

Green, J. M. and M. D. K. Owen. 2011. Herbicide-Resistant Crops: Utilities and limitations for herbicide-resistant weed management. J. Agric. Food Chem. 59: 5819-5829.

Haas, P. and F. Muller. 1987. Behaviour of glufosinate-ammonium in weeds. Pages 1075-1082 in Proceedings of the British Crop Protection Conference-Weeds.

Hartzler, R. G., B. A. Battles, and D. Nordby. 2004 Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. Weed Sci. 52:242-245.

Hausman, N. E., S. Singh, P. J. Tranel, D. E. Riechers, S. S. Kaundun, N. D. Polge, D. A. Thomas, and A. G. Hager. 2011. Resistance to HPPD-inhibiting herbicides in a population of waterhemp (*Amaranthus tuberculatus*) from Illinois, United States. Pest Manag. Sci. 67:258-261.

Heap, I. M. 2012. International survey of herbicide resistant weeds. http://www.weedscience.org. Accessed March 21, 2012.

Herman, P. L., M. Behrens, S. Chakraborty, B. M. Chrastil, J. Barycki, and D. P. Weeks. 2005. A three-component dicamba O-demethylase from *Pseudomonas maltophilia*, strain DI6: gene isolation, characterization, and heterozygous expression. J. Biol. Chem. 280:24759-24767.

Hess, F. D. 2000. Light-dependent Herbicides: An overview. Weed Sci. 48:160-170.

Hilton, H. W. 1957. Herbicide tolerance strain of weeds. Hawaiian Sugar Planters. Association. Annual Report. pp. 69.

Hirschberg J., A. B. Yehuda, I. Pecker, N. Ohad. 1987. Mutations resistant to Photosystem II herbicides. Pages 357-366 in: von Wettstein D, Chua N-M, Eds. Plant Molecular Biology. New York: Plenum Press.

Horak, M. J. and D. E. Peterson. 1995. Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol. 9:192-195.

Jalaludin, A., J. Ngim, B. Bakar, and Z. Alias. 2010. Preliminary findings of potentially resistant goosegrass (*Eleusine indica*) to glufosinate-ammonium in Malaysia. Weed Biol. Manag. 10:256-260.

Jha, P. 2008. Biology and ecology of Palmer amaranth (*Amaranthus pameri*). Ph. D. dissertation. Clemson, SC. Clemson Univ. 27 p.

Keeley, P. E., C. H. Carter, and R. J. Thullen. 1987. Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci. 35:199-204.

Kellogg, E. A. 1999. Phylogenetic aspects of the evolution of C4 photosynthesis. In R. F. Sage and R. K. Monson [eds.], C4 plant biology, 411–444. Academic Press, San Diego, California, USA.

Kiely, T., D. Donaldson, and A. Grube. 2004. Pesticide Industry Sales and Usage: 2000 and 2001 Market Estimates (EPA-733-R-04-001). In: Agency UEP, editor. Washington, DC.

Liphadzi, K. B. and J. A. Dille. 2006. Annual weed competitiveness as affected by preemergence herbicide in corn. Weed Sci. 54:15-165.

Massinga, R. A. and R. S. Currie. 2002. Impact of Palmer amaranth (*Amaranthus palmeri*) on corn (*Zea mays*) grain yield and yield and quality of forage. Weed Technol. 16:532-536.

Massinga, R. A., R. S. Currie, and T. P. Trooien. 2003. Water use and light interception under Palmer amaranth (*Amaranthus palmeri*) and corn competition. Weed Sci. 51:523-531.

Mersey, B. G., J. C. Hall, D. M. Anderson, and C. J. Swanton. 1990. Factors affecting the herbicidal activity of glufosinate-ammonium: absorption, translocation, and metabolism in barley and green foxtail. Pestic. Biochem. Physiol. 37:90-98.

Meyers, S. L., K. M. Jennings, J. R. Schultheis, and D. W. Monks. 2010. Interference of Palmer amaranth (*Amaranthus palmeri*) in sweetpotato. Weed Sci. 58:199-203.

Moore, J. W., D. S. Murray, and B. Westerman. 2004. Palmer amaranth (*Amaranthus palmeri*) effects on harvest and yield of grain sorghum (*Sorghum bicolor*). Weed Technol. 18:23-29.

Moran, G. R. 2005. (4-Hydroxyphenyl)pyruvate Dioxygenase. Archives of Biochemical and Biophysics. 433:117-128.

Nandula, V. K., C. H. Koger, J. A. Bond, T. W. Eubank, and R. C. Bond. 2011. Glyphosate-resistant waterhemp in Mississippi. Page 276 in Proceedings of the 64th Annual Meeting. San Juan, PR: Southern Weed Science Society.

Nandula. V. J., K. N. Reddy, S. O. Duke, and D. H. Poston. 2005. Glyphosate-resistant weeds: Current status and future outlook. DOI: 10.1564/16aug11.

Neto, F. S., H. D. Coble, and F. T. Corbin. 2000. Absorption, translocation, and metabolism of 14C-glufosinate in *Xanthium strumarium*, *Commelina diffusa*, and *Ipomoea purpurea*. Weed Sci. 48:171-175.

Neve, P. J. K. Norsworthy, K. L. Smith, I. A. Zelaya. 2011. Modeling glyphosate resistance management strategies for Palmer amaranth (*Amaranthus Palmeri*) in cotton. Weed Technol. 25:335-343.

Nordby, D. E. and R. G. Hartzler. 2004. Influence of corn on common waterhemp (*Amaranthus rudis*) growth and fecundity. Weed Sci. 52:255-259.

Norris, S. R., D. DellaPenna, and X. H. Shen. 1998. Complementation of the arabidopsis pds1 mutation with the gene encoding p-hydroxyphenylpyruvate dioxygenase. Plant Phys. 117:1317-1323.

Norsworthy, J. K., G. M. Griffith, R. C. Scott, K. L. Smith, and L. R. Oliver. 2008. Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) in Arkansas. Weed Technol. 22:108-113.

Perez, A. and M. Kogan. 2003. Glyphosate-resistant *Lolium multiflorum* in Chilean orchards. Weed Res. 43:12-19.

Peterson, J. and K. Hurle. 2001. Influence of climatic conditions and plant physiology on glufosinate-ammonium efficacy. Weed Res. 41:31-39.

Platzoldt, W. L., P. J. Tranel, A. G. Hager. 2005. A waterhemp (*Amaranthus tuberculatus*) biotype with resistance to herbicides targeting three unique sites of action. Weed Sci. 53:30-36

Powles, S. B. My view. 2003. Weed Sci. 51:471.

Powles, S. B., and C. Preston. 2006. Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance. Weed Technol. 20:282-289.

Jha, P., J. K. Norsworthy, M. J. Wilson, E. McCallister, J. DeVore, and J. Still. 2009. Influence of late-season herbicide applications on population dynamics of glyphosate-resistant Palmer amaranth biotypes. Page 7 in Proceedings of the 13th research conference. Fayetteville, AR: Arkansas Crop Protection Association.

Price, A. J. K. S. Balkcom, S. A. Culpepper, J. A. Kelton, R. L. Nicholas, and H. Schomberg. 2011. Glyphosate-resistant Palmer amaranth: A threat to conservation tillage. J. Soil Water Cons. 66:265-275.

Salas, R. A., F. E. Dayan, Z. Pan, S. B. Watson, J. W. Dickson, R. C. Scott, and N. R. Burgos. 2012. *EPSPS* gene amplification in glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) from Arkansas. Pest. Manag. Sci. 64:319-325.

Scott, B and K. L. Smith. 2010. Prevention and control of glyphosate-resistant pigweed in soybean and cotton. http://www.uaex.edu/Other_Areas/publications/PDF/FSA-2152.pdf. Accessed September 16th, 2012.

Sellers, B. A., R. J. Smeda, and J. Li. 2004. Glutamine synthetase activity and ammonium accumulation is influenced by time of glufosinate application. Pestic. Biochem. Physiol 78:9-20.

Shaner, D. L. 2000. The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. Pest Man. Sci. 56:320-326.

Simth, D. T., R. V. Baker, G. L. Steele. 2000. Palmer amaranth (*Amaranthus palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). Weed Technol. 14:122-126.

Sprague, C. L., E. W. Stoller, L. M. Wax, and M. J. Horak. 1997 Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) resistance to selected ALS-inhibiting herbicides. Weed Sci. 45:192-197.

Stuebler, H., H. Kraehmer, M. Hess, A. Schulz, C. Rosinger, C. 2008. Global changes in crop production and impact trends in weed management-an industry view. Page 309-319 in Proceedings of the 5th annual meeting. Vancouver, BC: Int. Weed Sci. Cong.

Tranel, P. J. and T. R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? Weed Sci. 50:700-712.

Tranel, P. J., J. J. Wassom, M. R. Jeschke, and A. L. Rayburn. 2002. Transmission of herbicide resistance from a monoecious to a dioecious weedy Amaranthus species. Theor. Appl. Genet. 105:674-679.

Tu, M., C. Hurd, and J. M. Randall. 2001. Weed control methods handbook: Tool and techniques for use in natural areas. The Nature Conservancy. http://www.invasive.org/gist/products/handbook/10.24-D.pdf. Accessed: March 8, 2012.

US EPA, 2006. Registration eligibility document for dicamba and associated salts. Office of Pesticide Programs, p. 4. Washington, DC.

VanGessel, M. J. 2001 Glyphosate-resistant horseweed from Delaware. Weed Sci. 49:703-705.

Vila-Aiub, M. M., M. C. Balbi, P. E. Gundel, C. M. Ghersa, and S. B. Powles. 2007. Evolution of glyphosate-resistant johnsongrass (*Sorghum halepense*) in glyphosate-resistant soybean. Weed Sci. 55:566-571.

Waddington, M. A. and B. G. Young. 2006. Interactions of herbicides and adjuvants with AE 0172747 on postemergence grass control. Weed Sci. 61:108-115.

Warwick, S. I. 1991. Herbicide resistance in weedy plants: Physiology and population biology. Ecology and Systematic. 22:95-114.

Watkinson, A. R., R. P. Freckleton, R. A. Robinson, and W. J. Sutherland. 2000. Predictions of biodiversity response to genetically modified herbicide-tolerant crops. Science 289:1554-1557.

Wetzel, D. K., M. J. Horak, D. Z. Skinner, and P. A. Kulakow. 1999. Transferal of herbicide resistance traits from Amaranthus palmeri to Amaranthus rudis. Weed Sci. 47:538-543.

Wilcut, J. W., A. C. York, W. J. Grichar, and G. R. Wehtje. 1995. The biology and management of weeds in peanut (*Arachis hypogaea*). Pages 207-244 in H. E. Pattee and H. T. Stalker (eds.) Advances in Peanut Science. Am. Peanut Res. and Educ. Soc., Stillwater, OK.

Wilson, H., T. E. Hines, R. R. Bellinder, and J. A. Grande. 1985. Comparisons of HO-39866, SC-0224, paraquat, and glyphosate in no-till corn (*Zea mays*). Weed Sci. 33: 531-536.

Young, B. G. 2006. Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol. 20:301-307.

Zollinger, R. K. and J. L. Ries. 2001. Overcoming antagonism of postemergence sulfonylurea herbicides on yellow foxtail. Page 216 in Proceedings of the 56th Annual Meeting. Milwaukee, WI: North Central Weed Science Society.

CHAPTER III

Glyphosate-Resistant Palmer Amaranth Response to Tembotrione, 2,4-D and Dicamba

Abstract

Glyphosate-resistant Palmer amaranth has become a major weed problem in upland areas in the southern US. Greenhouse experiments were conducted to evaluate twelve glyphosate-resistant (GR) Palmer amaranth populations for their tolerance to tembotrione, 2,4-D, and dicamba. Herbicide treatments were 0.25x, 0.5x, and 1x of 0.08 kg ha^{-1} tembotrione and 1x and 2x of 1.06 and 0.56 kg ha^{-1} for 2,4-D and dicamba, respectively. Tembotrione applied at 1x rate controlled Palmer amaranth 80 to 100%, with Mis-C, STF-B and STF-C populations being the least sensitive. The survival of plants from populations treated with 1x tembotrione ranged from 1% in Lee-A to 51% in Mis-C populations. Tolerance to tembotrione was more frequent in STF-B, STF-C, and Mis-B where 45, 26, and 23%, respectively, of the plants surviving 1x rate had <50% injury. 2,4-D applied at 1x or 2x controlled GR Palmer amaranth 98 to 100%. 2,4-D had excellent control of all populations 21 d after application. The 1x rate of dicamba caused great injury, but some plants survived even at the 2x rate. Visible injury ranged from 84 to 100% and mortality ranged from 36 to 91% 21 d after treatment with 1x dicamba. The least sensitive populations to dicamba had at least 60% survivors, up to 8% of which showed ≤50% injury or less. Thus, glyphosate-resistant Palmer amaranth populations showed differential response to tembotrione, 2,4-D, and dicamba. Tembotrione and dicamba have a high risk of selecting for resistance in some populations used in this experiment.

Nomenclature: Dicamba; tembotrione; 2,4-D; Palmer amaranth, *Amaranthus palmeri*.

Key words: Differential tolerance, herbicide resistance, HPPD inhibitor, auxinic herbicides.

Introduction

The genus *Amaranthus* has many weedy species that infest crop fields worldwide. Of all the *Amaranthus* species, Palmer amaranth (*Amaranthus palmeri*) is the most prolific, competitive, and common weed in crop fields in the southern US (Klingaman and Oliver 1994). Some of the characteristics that make Palmer amaranth an excellent competitor include high reproductive potential, estimated at between 60,000 and 500,000 seeds m^{-2} (Sellers et al. 2003; Fast et al. 2009), good water-use efficiency, good light interception, good respiration even under stress, and fast growth rate of up to 1.5 cm d^{-1} (Norsworthy et al. 2008a; Garvey 1999). Palmer amaranth can reach heights of over 2 m in a favourable growing season (Fast et al. 2009). The evolution of resistance to commonly used herbicides like glyphosate makes Palmer amaranth an economic concern for both growers and researchers. Apart from reducing crop yields, Palmer amaranth also interferes with production and processing activities such as harvesting and ginning in cotton (*Gossypium hirsutum* L.). Smith et al. (2000) reported that the presence of Palmer amaranth in cotton fields increased the harvesting time 2 to 3.5-fold.

Of the herbicides currently labeled for Palmer amaranth control in the US, glyphosate is preferred because it has broad-spectrum activity, has no soil residual effects, and has low environmental and human risks (Duke and Powles 2009). This makes glyphosate an herbicide with combined characteristics that no other herbicide has provided so far. Since the advent of glyphosate-resistant (GR) crop technology, the herbicide has been applied alone or mixed with other herbicides, in corn (*Zea mays* L.) and soybean (*Glycine max* L.) production more than any other herbicide (Cox 2006). Most farmers today use mostly glyphosate for weed control during the entire cycle of a crop. It is not surprising therefore that resistance to glyphosate has evolved in some weed species including Palmer amaranth as a result of such a heavy selection pressure.

Although individual plants of a species are visually indistinguishable, genetic differences generally exist. These minor genetic variations are usually reflected further in the response of a population to herbicides and this may confer the inherent ability to tolerate some herbicides. This is especially true in populations with wide genetic diversity, like Palmer amaranth (Steckel 2007; Hunter 2000). The variability in plant response to a herbicide such as bromoxynil resulting from genetic variation has been reported in slender wild oat (*Avena barbata* Pott ex Link), godetia [*Clarkia williamsonii* (Durand & Hilg.) F.H.Lewis & M.E.Lewis], and wild oat (*Avena fatua* L.) (Price et al. 1985). Burgos et al. (2011) reported differential tolerance of weedy rice (*Oryza sativa* L.) to glyphosate where by 115 accessions segregated into six groups based on their response to the herbicide. Differential tolerance and resistance have been implicated in weed succession changes within glyphosate-resistant agroecosystems (Zelaya and Owen 2005). Differential response of *Amaranthus* species to glyphosate, fomesafen, atrazine, imazethapyr, and diphenyether has also been previously reported (Norsworthy et al. 2008b; Patzoldt et al. 2002). Palmer amaranth is dioecious and therefore allows for outcrossing with other genetically compatible species, which in turn, increases its genetic diversity. The chances of having individuals with distinct characteristics are even higher in Palmer amaranth than in other species because of its high fecundity. Resistance of weeds to herbicides other than glyphosate is also becoming common, hence the need to understand the biology and physiology of different biotypes. To find appropriate alternative herbicides for weed management, target species (in this case, Palmer amaranth) need to be characterized in terms of herbicide sensitivity. The objectives of this study were to evaluate the response of Palmer amaranth to different rates of, tembotrione, 2,4-D, and dicamba and to determine the frequency and level of tolerance of glyphosate-resistant Palmer amaranth populations to these alternative herbicides.

Materials and Methods

Greenhouse experiments were conducted between October 2010 and July 2011 at the Altheimer greenhouses, Fayetteville, Arkansas, to evaluate the response of 12 Palmer amaranth populations to tembotrione, 2,4-D, and dicamba. Palmer amaranth seeds of the populations evaluated in the study were collected from growers' fields in Mississippi, Lee, Lawrence, Phillips, Conway, St Francis, Prairie, and Poinsett counties, Arkansas (Table 1;

Figure 1). Palmer amaranth seeds were collected from 10 to 20 plants surviving at least two 0.84 kg ae ha^{-1} glyphosate applications in the 2009 growing season. Five hundred mg of seeds from each plant were thoroughly mixed to make a composite that was used for experimentation. Palmer amaranth seeds from the composite were planted in cellular trays (Redwayfeed Garden and Pet supply, 290 Briceland Rd, Reedway, CA 95560) using Sunshine premix soil (Sunshine premix #1®, Sun Gro Horticulture, 15831 NE 8th Street, Suite 100, Bellevue, WA 98008). The trays were placed in the greenhouse with 34/26(±4) ^{o}C day/night temperatures and watered when needed to facilitate germination and growth of the seedlings. At the two-leaf stage, Palmer amaranth seedlings were thinned to one plant per cell. When Palmer amaranth seedlings were about 7-cm tall, tembotrione (Laudis®, 419 g ai L^{-1}, Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA), 2,4-D (Weedar 64®, 455 g ae L^{-1}, Nufarm Inc, 150 Harvester Drive, Burr Ridge, IL, 60527, USA), or dicamba (Clarity®, 479 g ae L^{-1}, Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA) were sprayed using a motorized boom equipped with two nozzles (TeeJet® 800067 flat-fan spray nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189) spaced 51 cm apart and calibrated to spray 187 L ha^{-1} of the solution at 262 kPa. The herbicides were applied at 1x and 2x of 1.06 and 0.56 kg ae ha^{-1} for 2,4-D and dicamba, respectively, and 0.25x, 0.5x, and 1x of 0.08 kg ai ha^{-1} for tembotrione. Ammonium sulfate (AMS) was added at 10 g L^{-1} to tembotrione. Methylated seed oil was also added to all tembotrione and 2,4-D treatments at 1% (v/v). The treated Palmer amaranth seedlings were returned to the greenhouse and maintained for 21 d when the evaluation was conducted.

The response of Palmer amaranth populations to the applied herbicides was evaluated in separate experiments. The experimental design for all experiments was randomized complete block with a split-plot treatment arrangement of herbicide rates (main plot) and populations (subplots). The herbicide rate and population combination were replicated four times. In each replicate, 25 plants per population were evaluated, giving a total of 100 plants at each run and 200 plants for the two runs that the experiments were conducted. Individual Palmer amaranth plants were visually assessed for injury 21 d after treatment (DAT) on a scale of 0 (no control) to 100% (complete control) relative to the nontreated control. Analysis of variance on injury and mortality data for each experiment was performed using PROC GLM and PROC GLIMMIX, respectively, in SAS (SAS®, v9.2, Statistical Analysis Systems Institute, SAS Circle, P.O. Box 8000, Cary, NC 25712-8000). Fisher's Protected LSD was used to separate rate, population, and interaction means, where necessary, at P = 0.05. Frequency analysis was also done to evaluate tolerance distribution of individual plants of the test populations to the labeled rates of the herbicides.

Results and Discussion

Tolerance of Palmer amaranth populations significantly differed within and across herbicide rates for tembotrione, 2,4-D and dicamba. The variability in responses of Palmer amaranth populations to tembotrione was greater at lower than at higher rates of tembotrione. The variability was narrower in 2,4-D and dicamba probably because both herbicides were applied at 1x or higher rates.

Palmer Amaranth Response to Tembotrione. No interactions involving population and herbicide rate were observed. The run effect was insignificant; therefore, the data was averaged

over runs. Differential response to tembotrione among Palmer amaranth populations was evident at all rates but was most common at lower rates of 0.25x and 0.5x. The three rates of tembotrione injured Palmer amaranth 12 to 30%, 60 to 83%, and 80 to 100%, respectively, 21 DAT (Table 2). All plants survived the 0.25x rate of tembotrione and only one population had >50% mortality at the 0.5x rate. At the 1x rate, Mis-B, STF-B and STF-C showed less injury than the other populations. The survival rate of plants at the 1x rate of tembotrione ranged from <1% in Lee-A to 51% in Mis-C populations (Table 3). Plants tolerant to the 1x rate were more frequent in STF-B, STF-C, and Mis-B where 45, 26, and 23%, respectively, of the survivors had <50% injury (Figure 2). Law-B, Law-C, Con-A, Cri-C, Pra-C, and STF-A had less than 10% of survivors with ≤50% injury. On the other hand, all survivors in Lee-A and Cri-C had ≥50% injury and are least likely to produce seeds. Populations STF-B, STF-C, and Mis-B would need additional control strategy to prevent seed production. Most treated plants of these tolerant populations had new growth with no injury symptoms 14 DAT. Bararpour et al. (2011) reported that 0.092 kg ha^{-1} tembotrione controlled Palmer amaranth 90 to 100% in corn. Lally (2011) reported similar results, where 0.092 kg ha^{-1} tembotrione controlled 100% of the susceptible Palmer amaranth 28 d after application in the greenhouse. In the same experiment, however, tembotrione at the same rate as above controlled 66 to 82% of the HPPD-resistant populations. In the field, Palmer amaranth control was lower (40 to 63%) than in the greenhouse (Lally 2011) probably because plants in the greenhouse attain the recommended size for spraying when they were still tender and therefore are more susceptible to herbicides than their counterparts in the field. Secondly, the herbicide was applied in the field study when plants were 15 to 27 cm; the tembotrione label recommends spraying the herbicide when *Amaranthus* species are <6-cm tall (Anonymous 2011). Having 18 to 27% of Mis-B, STF-C, STF-B survivors with less than 50% injury to the

labeled rate suggests that these populations have high proportions of tolerant plants that can be selected by tembotrione and constitute a 'new' resistant population.

Palmer Amaranth Response to 2,4-D. Due to minimal or no variance among replicates, the data were not subjected to analysis of variance. 2,4-D applied at 1x controlled all populations 99 to 100% (Table 2). Doubling the 2,4-D rate improved Palmer amaranth control to 100% in almost all populations. Both rates of 2,4-D provided excellent control 21 d after herbicide application (Table 2 and 3). The percent mortality ranged from 98 to 100% and was influenced by herbicide rate and Palmer amaranth population. The few surviving plants had at least 80% injury at the time of evaluation, and were unlikely to reproduce. Our findings are similar to those reported by Nandula et al. (2011) where 2,4-D controlled greater than 90% of GR *Amaranthus tuberculatus*, in Mississippi. However, less than 90% control of GR Palmer amaranth biotypes and spiny amaranth (*Amaranthus spinosus*) form 1.06 kg ha^{-1} 2,4-D has also been reported (Edwards 2011; Jha et al. 2009). In their experiments, 2,4-D provided better control of Palmer amaranth than did dicamba or glufosinate.

Palmer Amaranth Response to Dicamba. Herbicide rate by population interaction effect was observed on Palmer amaranth injury from dicamba. However, the rate by population interaction was not significant for mortality ($P = 0.1371$). In response to dicamba applied at 1x, Palmer amaranth populations had 84 to 100% injury 21 DAT (Table 2). However, some plants showed signs of recovery. The application of 2x dicamba significantly increased control of the less sensitive populations. However, none of the populations had 100% mortality even at the 2x rate. The mortality was 36 to 97%, with Pra-C and STF-A being the least sensitive, with at least 60% survivors, of which 8 and 2%, respectively, had ≤50% injury (Table 3; Figure 3). On the other hand, 11% of treated plants from Con-A survived 1x dicamba, 16% of which had <50% injury.

Other control measures need to be deployed to prevent these survivors from producing seeds and replenishing the seedbank with increasing numbers of tolerant plants and eventually selecting for a resistant biotype. Some populations having relatively more individuals with specific inherent resistance to a particular herbicide have higher chances of developing resistance, hence the need to have effective control strategies (Ozair 2008; Tharayil-Santhakumar 2003). Different responses to dicamba have been reported by other researchers. Doherty et al. (2010) reported 99% control of 7.5- and 15- cm GR Palmer amaranth by 0.58 kg ha^{-1} dicamba while Johnson (2011) reported a wide range of dicamba activity on Palmer amaranth from 61 to 97%. In other species, reduced sensitivity to dicamba is primarily due to genetic mutation(s). Resistance to dicamba in wild mustard (*Sinapis arvensis* L.) is due to a single dominant gene (Jaseniuk et al. 1995; Jugulam et al. 2005); a single recessive gene in yellow starthistle (*Centaurea solstitialis* L.); and additive genes in common hempnettle (*Galeopsis tetrahit* L.). Reduced absorption and increased herbicide detoxification are the only physiological factors that have been confirmed to endow resistance to dicamba (Weinberg et al. 2006). The mechanism of resistance to auxinic herbicides including dicamba has not been established in any of the other species (Preston et al. 2009).

Our experiments demonstrated that differential response to tembotrione, 2,4-D, and dicamba among the glyphosate-resistant Palmer amaranth populations exists. Among the populations, Pra-C seems to have higher tolerance to both 2,4-D and dicamba. In general, our data suggest that only a small percentage of glyphosate-resistant Palmer amaranth evaluated in this experiment can survive the labeled rate of 2,4-D and dicamba. However, dicamba should be applied at 2x rate or in combination with other strategies in order to kill more of the survivors having less than 50% injury when 1x dicamba is applied. Selection to dicamba would easily

occur given the above scenario. Similarly, tembotrione would need increased rates, or sequential application, or herbicide mixture to maximize control. Of the three herbicides, tembotrione has the highest risk of selecting for resistant population because of its high tendency to leave plants that can still reproduce.

Literature Cited

Anonymous. 2011. Laudis label. http://fs1.agrian.com/pdfs/Laudis_Herbicide_Label4b.pdf. Accessed March 2012.

Bararpour, M. T., L. R. Oliver, and C. G. Bell. 2011. Comparison of HPPD inhibitors for weed control programs in corn. Page 16 in Proceedings of the 64th annual meeting. San Juan, PR: Southern Weed Science Society.

Burgos, N. R., V. K. Shivrain, R. C. Scott, A. Mauromoustakos, Y. Kuk, M. A. Sales, J. Bullington. 2011. Differential tolerance of weedy red rice (*Oryza sativa* L.) from Arkansas, USA to glyphosate. Crop Prot. 30:986-994.

Corbett, J. L., S. D. Askew, W. E. Thomas, and J.W. Wilcut. 2004. Weed efficacy evaluations for bromoxynil, glufosinate, glyphosate, pyrithiobac, and sulfosate. Weed Technol. 18:443-453.

Cox, C. 1996. Herbicide factsheet: Glufosinate. J. Pest. Reform. 16:15-19.

Culpepper A. S., A. C. York, S. M. Brown, W. W. Hanna, J. W. Davis, W. K. Vencill, T. L. Grey, T. M. Webster, and J. M. Kichler. 2006. Glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) confirmed in Georgia. Weed Sci. 54:620-626.

Culpepper, A. S., A. C. York, R. B. Batts, and K. M. Jennings. 2000. Weed management in glufosinate- and glyphosate-resistant soybean (*Glycine max*). Weed Technol. 14:77-88.

Doherty, R. C., K. L. Smith, J. A. Bullington, and J. R. Meier. 2010. Palmer amaranth and *Ipomoea* species control with dicamba and glufosinate as influenced by weed size and herbicide rate. Page 101 in Proceedings of the 63rd annual meeting. Little Rock, AR: Southern Weed Science Society.

Drake, D. M., L. R. Oliver, and M. T. Barapour. 2009. Palmer amaranth (*Amaranthus Palmeri*) control in soybean. Page 13 in Proceedings of the 13th research conference. Fayetteville, AR: Arkansas Crop Protection Association.

Duke, S. O. and S. B. Powles. 2009. Glyphosate-resistant crops and weeds: Now and in the future. AgBioForum 12:346-357.

Edwards, E. M. 2010. Spiny amaranth control and aminopyralid persistence in Kentucky pastures. M. Sc. Thesis. Lexington, KY. University of Kentucky. 19p.

Everman, W. J., W. E. Thomas, J. W. Wilcut, and C. H. Koger. 2006. Weed control and physiological behavior with selected herbicides as influenced by rainfastness. Page 96 in Proceedings of the 59th annual meeting. San Antonio, TX: Southern Weed Science Society.

Fast, B. J., S. W. Murdock, R. L. Farris, J. B. Willis, and D. S. Murray. 2009. Critical timing of Palmer amaranth (*Amaranthus palmeri*) removal in second-generation glyphosate-resistant cotton. J. Cotton Sci. 13:32-36.

Gardner, A. P. 2006. Weed management in glufosinate-tolerant cotton (*Gossypium hirsutum* L.). M. Sc. Thesis. Raleigh, NC. North Carolina State University. 72-73 p.

Garvey, P. V. 1999. Goosegrass (*Eleusine indica*) and Palmer amaranth (*Amaranthus palmeri*) interference in plasticulture tomato. Ph.D. Dissertation. Raleigh, N.C.: North Carolina State University, 101 p.

Heap, I. M. 2010. International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: December 15, 2011.

Hill, A. S., E. C. Murdock, and A. Keeton. 1997. Weed control in Liberty Link® corn and soybeans. Pages 58-59 in Proceedings of the 50th Annual meeting. Houston, TX: Southern Weed Science Society.

Jasieniuk, M. J., I. N. Morrison, and A. L. Brule´-Babel. 1995. Inheritance of dicamba resistance in wild mustard (*Brassica kaber*). Weed Sci. 43:192-195.

Johnson, A. V. 2011. Weed and crop response to sub-lethal rates of dicamba, glufosinate, and 2,4-D. M. Sc. Thesis. Raleigh, NC. University of North Carolina. 97p.

Jugulam, M., M. D. McLean, and J. C. Hall. 2005. Inheritance of picloram and 2,4-D resistance in wild mustard (*Brassica kaber*). Weed Sci. 53:417-423.

Klingaman, T. E., and L. R. Oliver. 1994. Palmer amaranth (*Amaranthus palmeri*) interference in soybeans (*Glycine max*). Weed Sci. 42:523-527.

Lally, G. N. 2011. Pyrasulfotole and bromoxynil response in grain sorghum. M. Sc. Thesis. Manhattan, KA. Kansas State University. 87 p.

Main, C. L. and M. A. Jones. 2007. Glyphosate resistant Palmer amaranth in South Carolina. Pages 566 in Proceedings of the Beltwide Cotton Conferences. Memphis, TN: National Cotton Council of America.

Nandula, V. K., C. H. Koger, J. A. Bond, T. W. Eubank, and R. C. Bond. 2011. Glyphosate-resistant waterhemp in Mississippi. Page 276 in Proceedings of the 64th Annual Meeting. San Juan, PR: Southern Weed Science Society.

Norsworthy, J. K., G. M. Griffith, R. C. Scott, K. L. Smith, and L. R. Oliver. 2008a. Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) in Arkansas. Weed Technol. 22:108-113.

Norsworthy, J. K., R. C. Scott, K. L. Smith, and L. R. Oliver. 2008b. Response of Northeastern Arkansas Palmer amaranth (*Amaranthus palmeri*) accessions to glyphosate. Weed Technol. 22:408-413

Ozair, C.A. 2008. Herbicide-Resistance and Weed-Resistance Management. On line book "Herbicide-Resistance and Weed-Resistance Management". Weed Science Society of America. http://www.weedscience.org/paper/Book_Chapter_I.pdf. Accessed April 14, 2012

Patzoldt, W. L., P. J. Tranel, and A. G. Hager. Variable herbicide response among Illinois waterhemp (*Amaranthus rudis and tuberculatus*) populations. Crop Prot. 21:707-712.

Jha, P., J. K. Norsworthy, M. J. Wilson, E. McCallister, J. DeVore, and J. Still. 2009. Influence of late-season herbicide applications on population dynamics of glyphosate-resistant Palmer amaranth biotypes. Page 7 in Proceedings of the 13th research conference. Fayetteville, AR: Arkansas Crop Protection Association.

Preston, C., D. S. Belles, P. H. Westra, S. J. Nissen, and S. M. Ward. 2009. Inheritance of resistance to the auxinic herbicide dicamba in kochia (*Kochia scoparia*). Weed Science. 57:43-47.

Price, A. J., K. S. Balkcom, S. A. Culpepper, J. A. Kelton, R. L. Nichols, and H. Schomberg. 2011. Glyphosate-resistant Palmer amaranth: A threat to conservation tillage. J. Soil and Water Conserv. 66:265-275.

Price, S. C., R. W. Allard, J. E. Hill, and J. Naylor. 1985. Association between discreet genetic loci and genetic variability for herbicide reaction in plant populations. Weed Sci. 33:650-653.

Sellers, B. A., R. J. Smeda, W. G. Johnson, J. A. Kendig, and M. R. Ellersieck. 2003. Comparative growth of six Amaranthus species in Missouri. Weed Sci. 51:329-333.

Smith, D. T., G. L. Steele, and R. V. Baker. 2000. Palmer amaranth (*Amaranthus Palmeri*) affects yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). Weed Technol. 14:122-126.

Steckel, L. E., C. L. Main, A. T. Ellis, and T. C. Mueller. 2008. Palmer amaranth (*Amaranthus palmeri*) in Tennessee has low level glyphosate resistance. Weed Technol. 22:119-123.

Tharayil-Santhakumar, N. 2003. Mechanism of Herbicide Resistance in Weeds. On line book "Mechanism of Herbicides Resistance in Weeds". Weed Science Society of America. http://www.weedscience.org/paper/Mechanism%20of%20Herbicide%20resistance.PDF. Accessed April 17, 2012.

Wienberg, T., G. R. Stephenson, M. D. McLean, and J. C. Hall. 2006. MCPA (4-chloro-2-ethylphenoxyacetate) resistance in hemp-nettle (*Galeopsis tetrahit* L.). J. Agric. Food Chem. 54:9126-9134.

Table 1. Glyphosate-resistant Palmer amaranth sampling sites, cropping history, and management practices, Arkansas, 2009.

Population	County	Cropping history and management practices
Mis-B	Mississippi	Roundup-ready (RR) soybean (SB), Roundup + Flexstar appl. 10+ years continuous SB
Mis-C	Mississippi	No information
Cri-C	Crittenden	RR cotton Sequence + Staple, followed by (fb) Roundup; continuous cotton
Lee-A	Lee	RR soybean 2 appl. Roundup 2007 & 2006 RR cotton
Law-C	Lawrence	2007 - RR soybean-wheat; Roundup applied; grain sorghum in 2008
Law-B	Lawrence	Planted to soybean in 2008
Phil-B	Phillips	Sequence + Staple, fb Roundup; continuous cotton
STF-B	St Francis	Continuous cotton 10+ years; applied roundup in 2008
STF-A	St Francis	RR cotton 2008; continuous cotton 10+ years
STF-C	St Francis	SB 2008, Dual + Roundup 26 oz fb gly 22 oz; fallow 2006 & 07
Con-A	Conway	No information available
Pra-C	Prairie	No information available

Table 2**.** Control of 12 Palmer amaranth populations 21 d after treatment with tembotrione, 2,4-D, and dicamba.

	Palmer amaranth control[a]						
	Tembotrione			2,4-D		Dicamba	
AMAPA Population	0.02[b]	0.04	0.08	1.06	2.12	0.56	1.12
	% control						
Con-A	12.1	73.8	95.3	99.5	100.0	97.4	98.9
Cri-C	12.3	72.2	97.0	99.5	100.0	99.3	98.9
Law-B	17.3	69.4	93.5	100.0	100.0	99.9	99.1
Law-C	11.5	83.5	96.9	99.7	100.0	99.4	98.6
Lee-A	23.6	81.9	99.6	100.0	100.0	99.8	98.8
Mis-B	29.6	77.3	87.8	99.3	100.0	97.1	98.7
Mis-C	15.1	69.1	89.0	100.0	100.0	98.6	98.1
Phi-B	14.5	71.2	92.7	99.5	100.0	98.9	98.9
Pra-C	12.3	62.8	95.1	99.2	99.9	79.6	98.2
STF-A	16.9	60.4	94.1	100.0	100.0	85.4	98.8
STF-B	13.7	75.3	80.0	99.5	100.0	98.4	98.4
STF-C	13.1	70.5	84.5	99.7	100.0	98.3	99.1
LSD (0.05)							
Within herbicide[c]		6.7		*		3.4	
Within herbicide and rate[d]		6.3		*		3.0	

[a] Injury based on visual rating on a scale of 0 (no control) to 100% (total death) 21 DAT.

[b] Tembotrione in kg ai ha^{-1}; 2,4-D and dicamba in kg ae ha^{-1}. 1x rates for tembotrione, 2,4-D, and dicamba are 0.08, 1.06, and 0.56 kg ha^{-1}, respectively.

[c] Fisher's Protected LSD to compare rate means at the same or different populations;

[d] LSD to compare population means within the same herbicide rate.

*The data for 2,4-D was not subjected to ANOVA due to minimal or no variance among replicates and runs.

Table 3. Mortality of 12 palmer amaranth populations 21 d after tembotrione, 2,4-D and dicamba treatments.

	Palmer amaranth mortality[a]						
	Tembotrione			2,4-D		Dicamba	
AMAPA Population	0.02[b]	0.04	0.08	1.06	2.12	0.56	1.12
	% mortality						
Con-A	0	38.5	73.0	97.9	100.0	89.3	97.1
Cri-C	0	32.5	85.5	97.9	99.5	89.0	95.6
Law-B	0	37.5	63.5	99.7	100.0	93.9	95.6
Law-C	0	47.0	82.5	99.0	100.0	88.5	94.1
Lee-A	0	46.5	97.5	100.0	100.0	91.0	94.6
Mis-B	0	43.0	70.0	97.0	99.7	72.8	92.5
Mis-C	0	34.0	49.0	99.7	100.0	73.4	93.1
Phi-B	0	27.5	67.0	99.0	99.2	80.5	94.0
Pra-C	0	41.0	79.0	96.1	99.7	36.5	73.8
STF-A	0	20.0	70.0	99.7	100.0	36.0	86.0
STF-B	0	44.0	61.0	97.6	100.0	79.5	92.8
STF-C	0	54.0	63.5	99.2	100.0	79.0	93.5
LSD (0.05)							
Within herbicide[c]		6.8		0.5		6.8	
Within herbicide and rate[d]		13.6		*		13.6	

[a] Percent survival of plants treated with the herbicides 21 DAT.

[b] Tembotrione in kg ai ha^{-1}; 2,4-D and dicamba in kg ae ha^{-1}. 1x rates for tembotrione, 2,4-D, and dicamba are 0.08, 1.06, and 0.56 kg ha^{-1}, respectively.

[c] Fisher's Protected LSD to compare rate means at the same or different populations;

[d] LSD to compare population means within the same herbicide rate.

*The data for 2,4-D was not subjected to ANOVA due to minimal or no variance among replicates and runs.

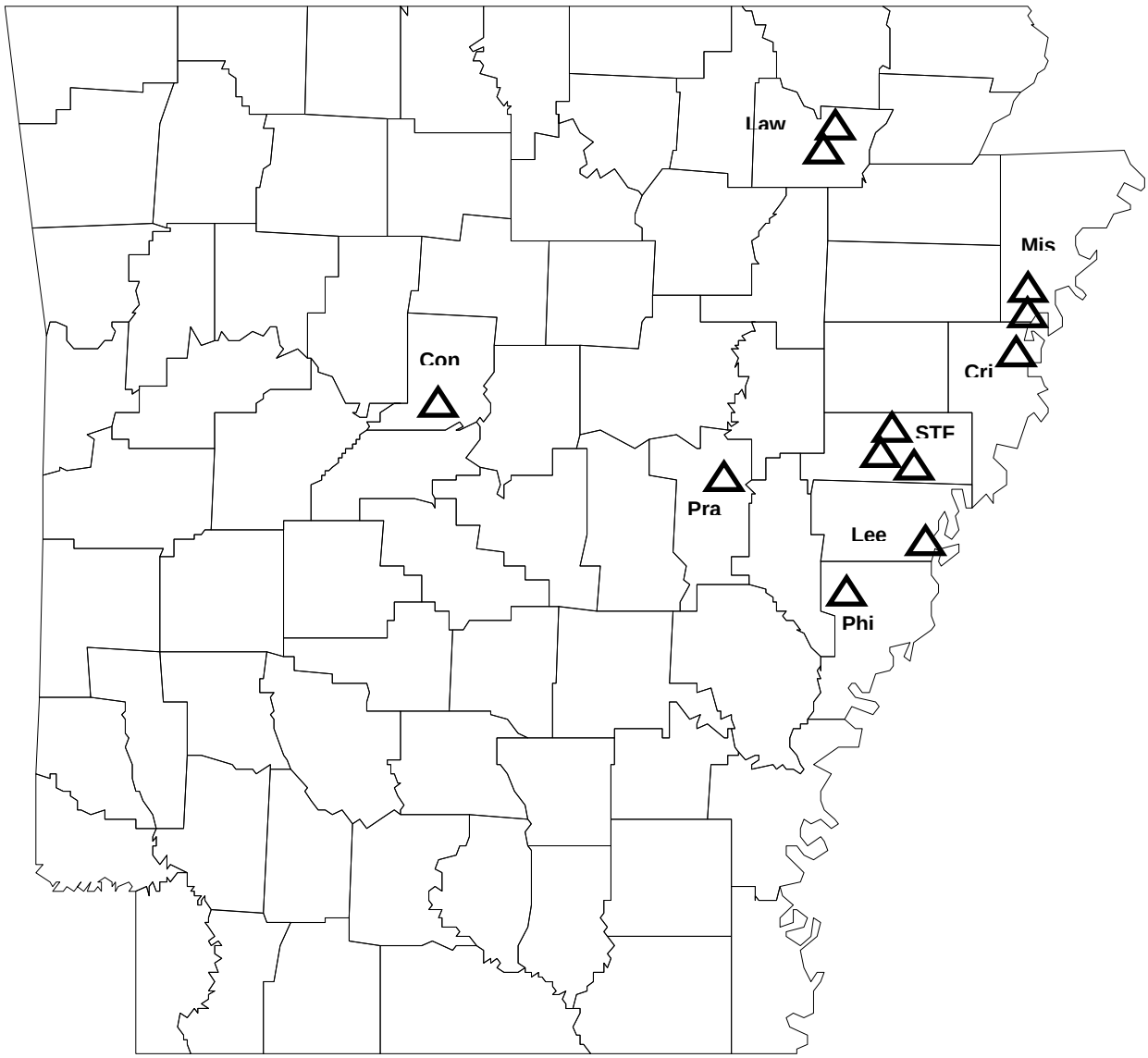

Figure 1. Arkansas map showing counties where Palmer amaranth populations were collected. [1]Abbreviation: Con = Conway, Law = Lawrence, Mis = Mississippi, Cri = Crittenden, STF = Saint Francis, Pra = Prairie, and Phil = Philips.

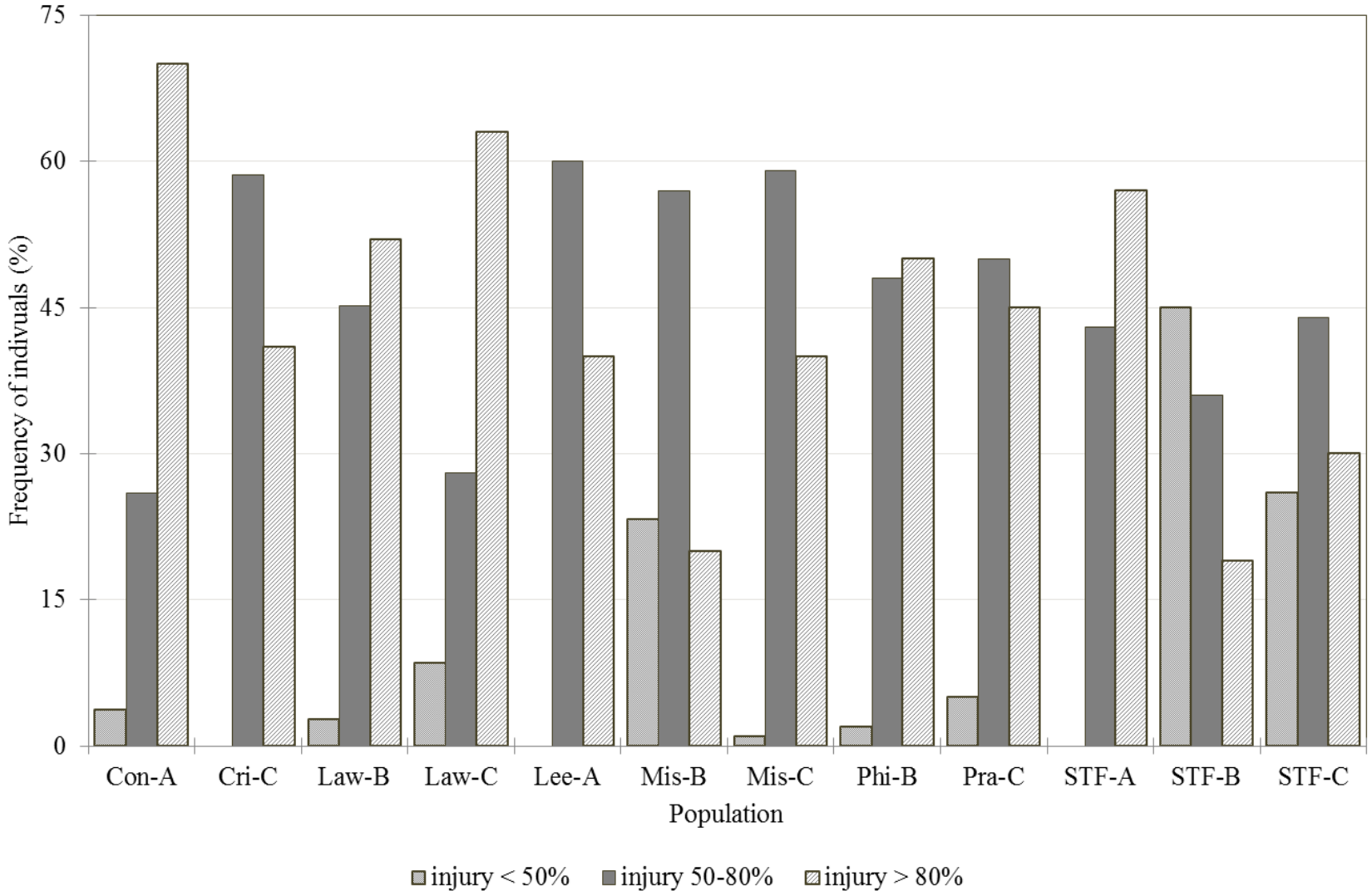

Figure 2. Injury frequency distribution of Palmer amaranth plants surviving 0.08 kg ai ha^{-1} tembotrione.

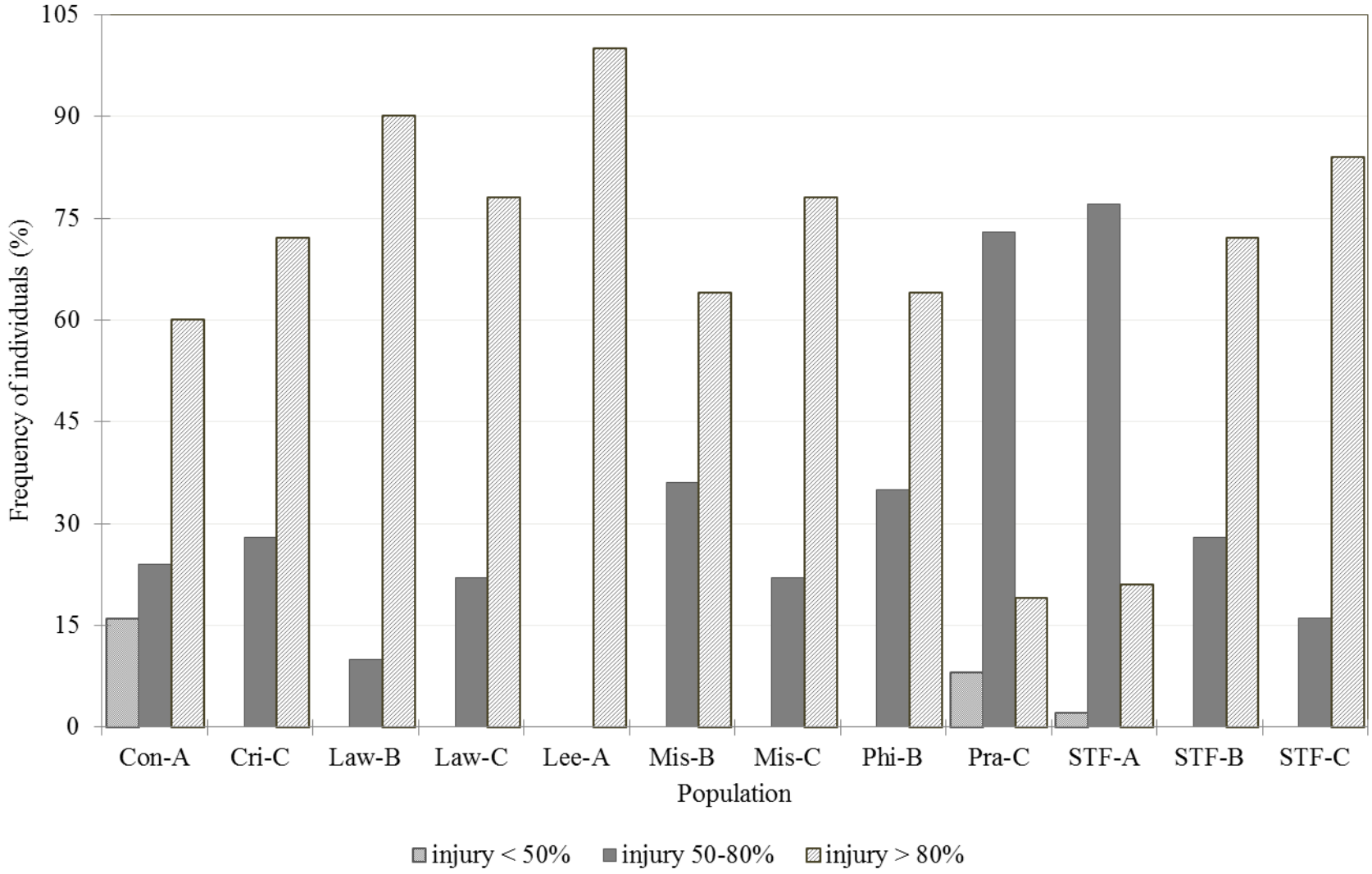

Figure 3. Injury frequency distribution of Palmer amaranth plants surviving 0.56 kg ae ha^{-1} dicamba.

CHAPTER IV

Glufosinate Mixed with Dicamba, Tembotrione or 2,4-D for Palmer Amaranth Control

Abstract

The use of herbicides with different modes of action is one of the recommended strategies for managing resistance in weeds. Greenhouse and field studies were conducted to evaluate the efficacy of glufosinate tank-mixed with dicamba, tembotrione or 2,4-D amine, and assess their potential interaction in the mixture. Glufosinate was evaluated at 0.25x, 0.5x, and 1x (0.73 kg ai ha^{-1}). The tank-mix options were 0.25x, 0.5x, and 1x (0.08 kg ai ha^{-1}) tembotrione; 0.25x, 0.5x, and 1x (1.12 kg ae ha^{-1}) 2,4-D; and 0.5x and 1x (0.56 kg ae ha^{-1}) dicamba. Interactions between herbicide in the mixtures were determined using the method described by Colby (1967). All herbicides applied individually at 1x rate controlled Palmer amaranth 99 to 100% in the greenhouse and 91 to 100% in the field. Glufosinate applied at 1x rate was as effective in controlling Palmer amaranth as when applied in combination with any rate of tembotrione, 2,4-D amine or dicamba. When half rates of 2,4-D and glufosinate were mixed, the control increased to 100% and was equal to glufosinate or 2,4-D applied alone or in a mixture at 1x. This study showed that half rates of 2,4-D and glufosinate can be applied, only in combination, without significantly compromising Palmer amaranth control. Based on the application of Colby's equation, the majority of glufosinate + tembotrione and some glufosinate + dicamba mixtures were not compatible; glufosinate + 2,4-D mixtures were generally additive and in few cases, synergistic. Reduced efficacy of herbicides was overcome by mixing 1x rates.

Nomenclature: Dicamba; glufosinate; tembotrione; 2,4-D; Palmer amaranth, *Amaranthus palmeri* S.Watts. AMAPA.

Key words: Tank mixing, HPPD, antagonism, synergism, field herbicide efficacy

Introduction

Over 80% of the world's total genetically modified crops' hectarage is planted to herbicide-resistant crops, basically with a single trait of glyphosate-resistance (GR) being utilized (Duke and Powles 2009). The tremendous adoption of GR crops resulted in increased use of glyphosate and reduced diversity of herbicides used for weed management. Extensive use of glyphosate has generated an intensive selection pressure resulting in the evolution of resistant biotypes in field bindweed (*Convolvulus arvensis* L.) (DeGennarro and Weller 1984), horseweed [*Conyza Canadensis* (L.) Cronq.] (Trainer et al. 2005), common ragweed (*Ambrosia artemisiifolia* L.) (Pollard et al. 2004), tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Patzoldt et al. 2004; Zelaya and Owen 2002), common waterhemp (*Amaranthus rudis* Benth.) (Light et al. 2011), Italian ryegrass (Perez et al. 2004; Perez-Jones et al. 2007), hairy fleabane [*Conyza bonariensis* (L.) Cronq.] (Dinelli et al. 2008), rigid ryegrass (*Lolium rigidum* Gaudin.) (Wakelin et al. 2004; Wakelin and Preston 2006), johnsongrass [*Sorghum halepense* (L.) Pers.] (Riar et al. 2011), goosegrass [*Eleusine indica* (L.) Gaertn.] (Mueller et al. 2011), annual bluegrass (*Poa annua* L.) (Brosnan et al. 2012), and Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Culpepper et al. 2006; Norsworthy et al. 2008). Twenty-one glyphosate-resistant weed species have been confirmed in 20 countries worldwide. The US has 13 glyphosate-resistant weed species. Horseweed, which occurs in 25 states, is the most widely occurring glyphosate-resistant weed species in the US, with Palmer amaranth in close second (Heap 2012). In Arkansas alone, glyphosate-resistant Palmer amaranth occurs in 30 counties, which include all counties in the eastern region and part of central Arkansas.

Herbicide resistance, defined as the inherited ability of a species to survive and reproduce following exposure to a rate of herbicide normally lethal to its wild type (WSSA 1998), usually

arises from selection of a small group of resistant plants from the existing large population (Duke et al. 1991). The evolution of resistance to herbicides is usually due to, among other factors, continuous application of the same herbicide, or different herbicides with the same mode of action. Resistance is also promoted by the use of herbicides with long residue period or application of herbicides with a highly specific site of action (Nosworthy et al. 2012; Ozair 2008; Tharayil-Santhakumar 2003). For example, the resistance of most weeds to glyphosate has largely been observed in places where there is extensive and exclusive glyphosate use over space and time (Culpepper et al. 2006; Perez-Jones et al. 2005; Pollard et al. 2004). Even with the most active herbicides, it is not always sustainable to have an economical and effective weed control from a single herbicide. Integration of different herbicides is therefore desirable to reduce or delay evolution of resistance. One management strategy for delaying the evolution of resistance is tank mixing herbicides. In addition to delaying or reducing the probability of resistance evolution in weeds, tank mixing herbicides broadens the weed spectrum controlled in one application and ensures season-long weed control if residual herbicides are included in the mixture (Bruff and Shaw 1992; Diggle et al. 2003; Norsworthy et al. 2012; Powles et al. 1997). Perhaps one other advantage of tank mixing over single-product application is to have a single application of herbicides that would otherwise need multiple applications, thereby saving time and resources. Tank mixing can lead to negative interaction between herbicides, which may reduce the activity of individual herbicides (Damalas 2004).

The resulting interaction between two or more herbicides can be additive, synergistic or antagonistic. Herbicides are considered to be additive if the control achieved by a mixture of two herbicides is similar to the expected sum of weed control. In situations where the observed activity from all the tank-mixed herbicides is greater or lower than the expected total activity if

the herbicides are applied separately, the herbicides are said to be synergistic or antagonistic, respectively (Hatzios and Penner 1985). Antagonism measures or defines the interaction between herbicides and not the agronomic usefulness of tank mixing. Therefore, an antagonistic mixture could sometimes still be useful for managing resistant weeds.

Not much is documented on the efficacy, let alone on the interaction of tank mixing glufosinate with tembotrione, dicamba or 2,4-D on Palmer amaranth control. Both increased and reduced activities in tank mixtures involving glufosinate, dicamba, or mesotrione with other herbicides have been reported. Chafin et al. (2010) found that GR Palmer amaranth control was less than 80% when glufosinate (0.47 kg ha^{-1}), 2,4-D (0.56, 0.84 or, 1.2 kg ha^{-1}), or dicamba (0.28, 0.56 or 1.1 kg ha^{-1}) were applied alone and the control was 25 to 32% higher when glufosinate was tank mixed with dicamba or 2,4-D. It was further observed that efficacy on broadleaf signalgrass (*Urochloa platyphylla* L.) was reduced by 8% when glufosinate was mixed with any of the rates of 2,4-D or dicamba. However, the efficacy of glufosinate plus dicamba or 2,4-D on carpetweed (*Mollugo verticillata* L.) remained unaffected (99%) at 28 d after treatment (DAT). In greenhouse experiments, Chuah et al. (2010) reported that five of the nine combinations of glufosinate and ametryn were synergistic whereas four were antagonistic. Antagonism was overcome when ametryn and glufosinate rates were increased from 12.5 or 25 to 80 g ha^{-1} and from 20 or 40 to 50 g ha^{-1}, respectively. Occurrence of antagonism was also reported when glufosinate was tank-mixed with atrazine for horseweed control in rye (*Secale cereale* L.) (Wilson et al. 1985). Dicamba antagonism of nicosulfuron activity on yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes] is reported to be common in North Dakota (Zollinger et al. 2001).

In addition to glufosinate-resistant crops, which were first commercialized in 1995, crops resistant to 2,4-D, dicamba, hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors, and possibly to the polyphenol oxidase (PPO) inhibiting herbicides are expected to be commercialized in the near future (Green et al. 2008; Green and Owen 2011; Herman et al. 2005; Stuebler et al. 2008). The advancements in these herbicide-resistant crops and increased use of the existing LibertyLink® technology will have an impact on GR weed management strategies in the southern US and elsewhere. Increased adoption of these crops and the subsequent increased use of dicamba, 2,4-D, tembotrione, and glufosinate are expected if the lessons from adoption of GR technology are to be the norm. Therefore, it is important that the efficacy of glufosinate, dicamba, tembotrione, and 2,4-D on Palmer amaranth be investigated. In light of the critical need to develop an effective solution for the control of GR Palmer amaranth, studies were conducted to determine if glufosinate tank-mixed with other herbicides can improve Palmer amaranth control while also evaluating the potential interaction between the mixtures.

Materials and Methods

Greenhouse Assay. Greenhouse experiments were conducted between June and August 2011 at the Altheimer greenhouses, Fayetteville, Arkansas. Pra-C population was used in this study because it showed 20% survival frequency to the labeled rate of glufosinate in the earlier studies (not published, data presented in Chapter III of this thesis). Palmer amaranth seeds were planted in a cellular tray with 50 cells (Redwayfeed Garden and Pet supply, 290 Briceland Rd, Reedway, CA 95560) filled with Sunshine premix soil (Sunshine premix #1®, Sun Gro Horticulture, 15831 NE 8th Street, Suite 100, Bellevue, WA 98008). The trays were placed in the greenhouse with 25/25(±6) ^{o}C day/night temperatures and watered when needed to facilitate seed germination and

growth of the seedlings. At the two-leaf stage, seedlings were thinned to allow growth of only one plant per cell. When Palmer amaranth seedlings were about 7-cm tall, glufosinate (Ignite 280 SL®, 280 g ai L^{-1}, Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA), tembotrione (Laudis®, 419 g ai L^{-1}, Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA), 2,4-D (Weedar 64®, 455 g ae L^{-1}, Nufarm Inc, 150 Harvester Drive, Burr Ridge, IL, 60527, USA), or dicamba (Clarity®, 479 g ae L^{-1}, Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA) were sprayed using a motorized boom with two nozzles (TeeJet® 800067 flat-fan, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189, USA) spaced 51 cm apart. The rates of glufosinate (Factor A) were 0.25, 0.5, and 1x the recommended rate of 0.73 kg ai ha^{-1}. The tank-mix options (Factor B) were tembotrione at 0.25, 0.5, or 1x (0.08 kg ai ha^{-1}), dicamba at 0.5 or 1x (0.56 kg ae ha^{-1}) or 2,4-D at 0.25, 0.5 or 1x (1.06 kg ae ha^{-1}). Ammonium sulfate was added at 10 and 20 g L^{-1} to tembotrione- and glufosinate-alone tanks, respectively. Methylated seed oil was added to all tembotrione and 2,4-D treatments at 1% (v/v). No adjuvants were added to all tank mixtures. All treatments were applied at 276 kPa in 187 L ha^{-1} water. Dicamba and 2,4-D treated plants were placed in separate greenhouses for 24 h to avoid herbicide vapor drift to other treatments; thereafter, the plants were returned to the greenhouse where the tembotrione- and glufosinate-treated plants were kept. The plants were watered as needed and fertilized weekly for an additional 21-d period when herbicide efficacy was evaluated.

Field Experiment. The field experiment was conducted in 2012 at the Vegetable Research Station (VRS), Kibler, Arkansas. Experimental plots were 2.4 m^2, with 0.6- and 1.2-m alleys between plots and replicates, respectively. Palmer amaranth was hand-seeded 20 cm apart in the middle 1.2 m^2 leaving an additional 0.6-m border around the plot, giving a seedling density of 25

plants m^{-2}. The same rates and tank mixtures as in the greenhouse experiment were used. Herbicides were sprayed using a CO_2 backpack sprayer with 3-nozzle (TeeJet® 11002 VS, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189) spaced 51 cm apart and calibrated to deliver 187 L ha^{-1} at 248 kPa. The mean RH for the greenhouse and field experiments was 52 and 32%, respectively.

Experimental Design. Both greenhouse and field experiments were laid out in a randomized complete block design (RCBD) and treatments were replicated four times. The experiment was conducted twice in the greenhouse and once in the field. Palmer amaranth seedlings were visually rated for injury 21 DAT using a scale of 0 to 100% for no control and total death, respectively. The herbicide intercation was evaluated using the equation described by Colby (1967) shown below;

$$E_1 = (X_1 + Y_1) - (X_1Y_1 / 100) \qquad (1).$$

Where E_1 is the expected control from the combination of herbicides A and B; X_1 and Y_1 are the observed control from herbicide A and B alone, respectively. The expected control from the tank mixture was calculated using observed control values from individual herbicides used in the mixture within that particular replicate. The expected control (E_1) was then compared with the observed control obtained from the tank mix using Fisher's Protected LSD (Hicks et al. 1998; Koger et al. 2007). The interaction was considered to be synergistic if the observed plant response to the herbicide mixture was significantly higher than the expected, or antagonistic if the observed control was significantly lower than the expected, or additive if the observed and the expected responses were similar. The injury and mortality data were subjected to ANOVA using PROC GLM in SAS (SAS®, version 9.2, Statistical Analysis Systems Institute, SAS

Circle, P.O. Box 8000, Cary, NC 25712-8000) and means were separated by Fisher's Protected LSD at the 5% level of significance.

Results and Discussion

Response to Herbicides in the Greenhouse. Palmer amaranth control at 21 DAT ranged from 64 to 100% in the greenhouse. Generally, 0.5x and 1x rates of the herbicides, except for dicamba, provided similar Palmer amaranth control 21 DAT (Table 1). All herbicides applied individually at 1x rate were equally effective in controlling Palmer amaranth and injured palmer amaranth 99 to 100%. When 1x rates of individual herbicides were applied, Palmer amaranth mortality ranged from 91 to 100% (Table 2). Glufosinate applied at 1x rate was as effective in controlling Palmer amaranth as when applied in combination with any rate of tembotrione, 2,4-D amine or dicamba. Comparably, Chafin et al. (2010) and Culpepper et al. (2010) reported 73% Palmer amaranth control by 0.42 kg ha^{-1} glufosinate alone, 69 to 79% control by 2,4-D alone, and 91 to 96% control by tank-mixtures of glufosinate plus 2,4-D, regardless of 2,4-D rate.

Response to Herbicides in the Field. No differences between 0.5x and 1x rates of dicamba on Palmer amaranth control were observed but 1x rates of tembotrione, 2,4-D, and glufosinate provided significantly greater control than their respective 0.5x rates. The 1x rates of glufosinate and 2,4-D had similar Palmer amaranth control (91 and 93%) and was higher than the 1x rates of dicamba (83%) and tembotrione (69%) (Table 3). Although tembotrione applied at the 1x rate was as effective as glufosinate, 2,4-D, and dicamba in the greenhouse, it provided the least Palmer amaranth control in the field. The addition of any rate of 2,4-D or 1x rate of dicamba to glufosinate increased Palmer amaranth control by 4 to 10%; in contrast, the addition of a 1x rate of tembotrione resulted in only 2% increase in control. A mixture of 0.25x rates of 2,4-D and

glufosinate controlled >90% of Palmer amaranth. When half rates of 2,4-D and glufosinate were mixed, the control increased to 100% and was equal to glufosinate or 2,4-D applied alone or in a mixture at 1x.

Generally, the efficacy of 1x doses of glufosinate, dicamba, and tembotrione was lower in the field study than in the greenhouse, controlling Palmer amaranth 91, 94, and 92% respectively, probably because plants in the greenhouse reach the recommended size for herbicide application when they were still tender, hence more susceptible. On the other hand, the field environment is associated with heat stress, low relative humidity (RH) and sometimes low soil moisture content. Physiologically, these are preconditions for the synthesis of thicker leaf cuticles, which reduce herbicide absorption (Hatterman-Valenti et al. 2006; Hull et al. 1975; Monaco et al. 2002; Peregoy et al. 1990). The micro environment has also been found to affect absorption and translocation of herbicides in plants. Anderson et al. (1993) reported a reduction from total control at 95% RH to 30% growth inhibition at 40% RH in green foxtail (*Setaria viridis*). The mean RH for our greenhouse and field experiments was 52 and 32%, respectively.

Based on this study, half rates of 2,4-D and glufosinate may be used, only in combination, without compromising Palmer amaranth control. Reduced rates that are lethal to weeds are economical and environmental friendly, but they should be tested in a wide array of environments and geographies to test for robustness of performance of the mixture. Otherwise, if the mixture of reduced rates fails to attain 100% control, then selection for resistant plants ensues. Such mixture should also be used in conjunction with all other weed management practices.

Herbicide Antagonism. Tank mixing 1x rates of glufosinate with 0.25 or 0.5x rate of tembotrione antagonized the activity of each herbicide thereby reducing Palmer amaranth control

by 4 to 7% both in the greenhouse (Table 1) and in the field (Table 3). Antagonism was observed in 89% of the glufosinate + tembotrione mixtures in both experiments (Table 1 and Table 3). Antagonism was also observed in both experiments when <1x glufosinate rates were mixed with 0.5x rate of dicamba. In both cases, reduced control resulting from antagonism was overcome by increasing the rate of glufosinate, tembotrione or dicamba to the 1x rate. This approach has also been used by other researchers to overcome antagonism (Chuah et al. 2010; Lanclos et al. 2002). Herbicide interaction (synergistic or antagonistic) involving glufosinate with other herbicides in the tank mixture has been reported previously. Burke et al. (2005) found that the efficacy of clethodim applied at 105 or 140 g ha^{-1} on goosegrass control was antagonized by at least 52 percentage points when mixed with 290 or 410 g ha^{-1} glufosinate. Antagonism was also noted on johnsongrass, broadleaf signalgrass, fall panicum (*Panicum dichotomiflorum* Michx.), goosegrass, and large crabgrass [*Digitaria sanguinalis* (L.) Scop.], when 468 g ha^{-1} glufosinate was mixed with clethodim, fluazifop-P, quizalofop-P, or sethoxydim (Gardner et al. 2006).

This study shows that the majority of glufosinate + tembotrione and some glufosinate + dicamba tank-mixtures were not compatible. Based on the application of Colby's equation, 2,4-D and glufosinate tank mixtures were generally additive and in a few cases, synergistic. Glufosinate and 2,4-D combination at 0.5x and 1x rates controlled Palmer amaranth 100%. On the other hand, tembotrione and glufosinate tank mixes in this experiment were not beneficial and the combination of the two herbicides did not provide additional control over separate herbicide applications. Thus, mixing tembotrione and glufosinate is not advisable to growers.

Literature Cited

Anderson, D. M., C. J. Swanton, J. C. Hall, and B. G. Mersey. The influence of temperature and relative humidity on the efficacy of glufosinate-ammonium. Weed Res. 33:139-147.

Brosnan, J. T., G. K. Breeden, and T. C. Mueller 2012. A glyphosate-resistant biotype of annual bluegrass in Tennessee. Weed Sci. 60:97-100.

Bruff, S. A. and D. R. Shaw. 1992. Tank-mix combinations for weed control in stale seedbed soybean (*Glycine max*). Weed Technol. 6:45-51.

Burke, I.C., S.D. Askew, J.L. Corbett and J.W. Wilcut. 2005. Glufosinate antagonizes clethodim control of goosegrass (*Eleusine indica*). Weed Technol. 19:664-668.

Chafin, J. E., A. S. Culpepper, and L. B. Braxton. 2010. Palmer amaranth, benghal dayflower, carpetweed, pitted morningglory, and broadleaf signalgrass response to glufosinate applied alone or mixed with 2,4-d or dicamba. Beltwide Cotton Conferences, New Orleans, Louisiana. National Cotton Council of America.

Chuah, T. S., L. V. Lun, C. T. San, and I. B. Sahid. 2010. Initial report of glufosinate and paraquat multiple resistance that evolved in a biotype of goosegrass (*Eleusine indica*) in Malaysia. Weed Biol. Manag. 10:229-233.

Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide concentrations. Weeds 15:20-22.

Culpepper, A. S., J. E. Chafin, L. B. Braxton, and J. S. Richburg. 2010. Control of glyphosate-resistant Palmer amaranth in DHT cotton. Page 89 in Proceedings of the 63rd Annual meeting. Little Rock, AR. South. Weed Sci. Soc.

Culpepper, A. S., T. L. Grey, W. K. Vencill, J. M. Kichler, T. M. Webster, S. M. Brown, A. C. York, J. W. Davis, and W. W. Hanna. 2006. Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. Weed Sci. 54:620-626.

Damalas, C. A. 2004. Herbicide Tank Mixtures: Common Interactions. Int. J. Agric. Biol. 6:209-212.

DeGennaro, F. P. and S. C. Weller. 1984. Differential susceptibility of field bindweed (*Convolvulus arvensis*) biotypes to glyphosate. Weed Sci 32:472–476.

Diggle, A. J., P. B. Neve, and F. P. Smith. 2003. Herbicides used in combination can reduce the probability of herbicide resistance in finite weed populations. Weed Res. 43:371-382.

Dinelli, G., I. Marotti, P. Catizone, A. Bonetti, J. M. Urbano, and J. Barnes. 2008. Physiological and molecular basis of glyphosate resistance in *C. bonariensis* (L.) Cronq. biotypes from Spain. Weed Res. 48:257-265.

Duke, S. O. and S. B. Powles. 2009. Glyphosate-resistant crops and weeds: Now and in the future. AgBioForum 12:346-357.

Duke, S. O., A. L. Christy, F. D. Hess, and Z. S. Holt. 1991. "Herbicide- resistant crops". Comments from CAST 1991-1, Council of Agri. Sci. and Technol. Ames, I.A.

Gardner, A. P., A. C. York, D. L. Jordan, and D. W. Monks. 2006. Glufosinate antagonizes postemergence graminicides applied to annual grasses and johnsongrass. J. Cotton. Sci. 10:319-327.

Hatterman-Valenti, H. M., A. Pitty, and M. D. K. Owen. 2006. Effect of environment on giant foxtail (*Setaria faberi*) leaf wax and fluazifop-P absorption. Weed Sci. 54:607-614.

Hatzios, K. K. and D. Penner. 1985. Interactions of herbicides with other agrochemicals in higher plants. Weed Sci. 1:1-63

Heap, I. M. 2012. Glycine resistant weeds by country and species. http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go. Accessed: March 29, 2012.

Hicks, T. V., G. R. Wehtje, and T. L. Grey. 1998. The interaction of pyridate and 2,4-DB in peanut (*Arachis hypogaea*), Florida beggarweed (*Desmodium tortuosum*), and sicklepod (*Senna obtusifolia*). Weed Sci. 46:284-288

Hull, H. M., H. L. Morton, and J. R. Wharrie. 1975. Environmental influences on cuticle development and resultant foliar penetration. Bot. Rev. 41:421-432.

Koger, C. H., I. C. Burke, D. K. Miller, J. A. Kendig, K. N. Reddy, and J. W. Wilcut. 2007. MSMA antagonizes glyphosate and glufosinate efficacy on broadleaf and grass weeds. Weed Technol. 21:159-165.

Light, G. G., M. Y. Mohammed, P. A. Dotray, J. M. Chandler, and R. J. Wright. 2011. Glyphosate-resistant common waterhemp (*Amaranthus rudis*) Confirmed in Texas. Weed Technol. 25:480-485.

Monaco, T. J., S. C. Weller, F. M. Ashton. 2002. Weed Science: Principles and Practicies. 4th ed. New York: John Willey and Sons Inc. 103p.

Mueller, T. C., K. A. Barnett, J. T. Brosnan, and L. E. Steckel Glyphosate-resistant goosegrass (*Eleusine indica*) confirmed in Tennessee. Weed Sci. 59:562-566.

Norsworthy, J. K., G. M. Griffith, R. C. Scott, K. L. Smith, and L. R. Oliver. 2008. Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol. 22:108-113.

Norsworthy, J. K., S. M. Ward, D. R. Shaw, R. S. Llewellyn, R. L. Nichols, T. M. Webster, K. W. Bradley, G. Frisvold, S. B. Powles, N. R. Burgos, W. W. Witt, and M. Barrett. 2012.

Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Sci. 60:31-62.

Ozair, C.A. 2008. Herbicide-Resistance and Weed-Resistance Management. On line book "Herbicide-Resistance and Weed-Resistance Management". Weed Science Society of America. http://www.weedscience.org/paper/Book_Chapter_I.pdf. Accessed April 14, 2012.

Patzoldt, W. L., P. J. Tranel, and A. G. Hager. 2002. Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. Crop Prot. 21:707-712.

Peregoy, R. S., L. M. Kitchen, P. W. Jordan, and J. L. Griffin. 1990. Moisture stress effects on the absorption, translocation, and metabolism of haloxyfop in johnsongrass (*Sorghum halepense*) and large crabgrass (*Ditaria sanguinalis*). Weed Sci. 38:331-337.

Perez, A., C. Alister, and M. Kogan. 2004. Absorption, translocation and allocation of glyphosate in resistant and susceptible Chilean biotypes of *Lolium multiflorum*. Weed Biol. Manag. 4:56-58.

Perez-Jones, A., K. W. Park, J. Colquhoun, C. Mallory-Smith, and D. Shaner. 2005. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. Weed Sci. 53:775-779.

Pollard, J. M., B. A. Sellers, and R. J. Smeda. 2004. Differential response of common ragweed to glyphosate. Page 145 in Proceedings of the 59th Annual Meeting. Champaign, IL. North. Cent. Weed Sci. Soc.

Powles, S. B., C. Preston, I. B. Bryan, and A. R. Jutsum. 1997. Herbicide resistance: impact and management. Adv. Agron 58:57-93.

Riar, D. S., J. K. Norsworthy, D. B. Johnson, R. C. Scott, and M. Bagavathiannan 2011. Glyphosate resistance in a Johnsongrass (*Sorghum halepense*) biotype from Arkansas. Weed Sci. 59:299-304.

Sankula, S., M. P. Braverman, and S. D. Linscombe. 1997. Glufosinate-resistant, BAR-transformed rice (*Oryza sativa*) and red rice (*Oryza sativa*) response to glufosinate alone and in mixtures. Weed Technol. 11:662-666.

Tharayil-Santhakumar, N. 2003. Mechanism of Herbicide Resistance in Weeds. On line book "Mechanism of Herbicides Resistance in Weeds". Weed Science Society of America. http://www.weedscience.org/paper/Mechanism%20of%20Herbicide%20resistance.PDF. Accessed April 17, 2012.

Wakelin, A. M. and C. Preston. 2006. Inheritance of glyphosate resistance in several populations of rigid ryegrass (*Lolium rigidum*) from Australia. Weed Sci 54:212-219.

Wakelin, A. M., D. F. Lorraine-Colwill, and C. Preston. 2004. Glyphosate resistance in four different populations of *Lolium rigidum* as associated with reduced translocation of glyphosate to meristematic zones. Weed Res 44:453-459.

Wilson, H., T. E. Hines, R. R. Bellinder, and J. A. Grande. 1985. Comparisons of HO-39866, SC-0224, paraquat, and glyphosate in no-till corn (*Zea mays*). Weed Sci. 33:531-536.

WSSA. 1998. Resistance and tolerance definitions. Weed technol. 12:789.

Zelaya, I. A. and M. D. K. Owen. 2002.*Amaranthus tuberculatus* (Mq ex DC) J.S. Sauer: Potential for selection of glyphosate resistance. Pages 630–633 in Proceedings of the 13th Annual Australian Weed Conference. Perth, Australia: Plant Protection Society of WA.

Zollinger, R. K. and J. L. Ries. 2001. Overcoming antagonism of postemergence sulfonylurea herbicides on yellow foxtail. Page 216 in Proceedings of the 56th Annual Meeting. Milwaukee, WI. North Cent. Weed Sci. Soc.

Table 1. Palmer amaranth control at 21 DAT with glufosinate plus tembotrione, 2,4-D or dicamba in the greenhouse, Fayetteville, Arkansas, 2011.

Tank-mix option		Palmer amaranth control[b]			
		Glufosinate[c]			
Herbicide	Rate[a]	0	0.18	0.37	0.73
	kg ha^{-1}	----------%----------			
None	0.00	0.0	69.8	93.7	99.4
Tembotrione	0.02	64.1	65.5 (90.9)-[*]	78.5 (99.1)-	92.1 (99.9)-
Tembotrione	0.04	93.4	89.6 (98.4)-	96.8 (99.8)-	93.8 (100.0)-
Tembotrione	0.08	97.9	96.8 (99.6)-	99.7 (100.0)-	99.6
2,4-D	0.27	88.3	98.6	99.9	100.0
2,4-D	0.53	96.8	98.9	100.0	100.0
2,4-D	1.06	99.8	100.0	100.0	100.0
Dicamba	0.28	92.8	95.0 (98.0)-	96.0 (99.7)-	100.0
Dicamba	0.56	99.6	99.8	99.9	100.0
[d]$LSD_{0.05}$		----------5.5----------			

[a] Tembotrione rate in kg ai ha^{-1}; 2,4-D and dicamba rates in kg ae ha^{-1}.

[b] Injury based on visual rating 21 DAT on a scale of 0 to 100%; 0 = no injury, 100 = total death. n = 200.

[c] Glufosinate rate in kg ai ha^{-1}.

[d] Fisher's Protected LSD to compare treatment means within the same or across columns.

[*] Values in parentheses, calculated by Colby (1967) method using the raw data and not the means in the table, indicate the expected activity from tank mixtures having significant differences between the observed and the expected at P = 0.05. A negative sign (-) denotes an antagonistic response.

Table 2. Mortality of Palmer amaranth at 21 DAT following glufosinate plus tembotrione, 2,4-D or dicamba tank-mix applications in the greenhouse, Fayetteville, Arkansas, 2011.

Tank-mix option		Palmer amaranth control[b]			
		Glufosinate			
Herbicide	Rate[a]	0[c]	0.18	0.37	0.73
	kg ha^{-1}	----------%----------			
None	0.00	0	49	77	98
Tembotrione	0.02	37	39	57	77
Tembotrione	0.04	82	78	90	81
Tembotrione	0.08	91	91	98	98
2,4-D	0.27	78	94	99	100
2,4-D	0.53	88	95	100	100
2,4-D	1.06	100	100	100	100
Dicamba	0.28	80	75	77	100
Dicamba	0.56	96	99	99	100
[d]$LSD_{0.05}$		----------10----------			

[a] Tembotrione rate in kg ai ha^{-1}; 2,4-D and dicamba rates in kg ae ha^{-1}.

[b] Mortality based on death count 21 DAT calculated as dead plants/total plants sprayed, expressed as %.

[c] Glufosinate rate in kg ai ha^{-1}.

[d] Fisher's Protected LSD to compare treatment means within the same or across columns.

Table 3. Palmer amaranth control 21 DAT following treatment with glufosinate plus tembotrione, 2,4-D or dicamba, Kibler, Arkansas, 2012.

Tank-mix option		Palmer amaranth control[b]			
		Glufosinate			
Herbicide	Rate[a]	0[c]	0.18	0.37	0.73
	kg ha^{-1}	----------%----------			
None	0.00	0.0	38.3	83.8	90.9
Tembotrione	0.02	17.6	52.7	55.7 (86.7)-	60.1 (93.1)-
Tembotrione	0.04	59.4	55.7 (86.7)-[*]	73.2 (94.3)-	77.7 (96.7)-
Tembotrione	0.08	69.4	60.1 (93.2)-	93.3 (97.0)-	91.5 (98.5)-
2,4-D	0.27	43.3	79.9 (64.7)+	87.6	94.4
2,4-D	0.53	75.6	95.8 (84.4)+	100.0	100.0
2,4-D	1.06	93.3	91.7	100.0	100.0
Dicamba	0.28	83.3	66.7 (88.9)-	81.3 (94.3)-	94.4
Dicamba	0.56	83.3	76.4 (87.5)-	93.3	100.0
[d]$LSD_{0.05}$		----------2.8----------			

[a] Tembotrione rate in kg ai ha^{-1}; 2,4-D and dicamba rates in kg ae ha^{-1}.

[b] Mortality based on death count 21 DAT calculated as dead plants/total plants sprayed, expressed as %.

[c] Glufosinate rate in kg ai ha^{-1}.

[d] Fisher's Protected LSD to compare treatment means within the same or across columns.

[*] Values in parentheses, calculated by Colby (1967) method using the raw data and not the means in the table, indicate the expected activity from tank mixtures having significant differences between the observed and the expected at P = 0.05. A negative sign (-) denotes an antagonistic response.

CHAPTER V

Palmer Amaranth Response and Tolerance Mechanism to Glufosinate

Abstract

Twelve Palmer amaranth populations were screened for tolerance using 0.25 to 1x of 0.73 kg ha^{-1} glufosinate in the greenhouse. Glufosinate applied at 0.25x, 0.5x, and 1x injured Palmer amaranth populations 13 to 67%, 49 to 95%, and 94 to 100%, respectively, 21 d after application. Although, 29% of Pra-C plants survived the 1x rate, only 13% of survivors had <75% injury, implying that their survival to reproduction stage was unlikely. However, the 2% of Pra-C, 1% of STF-C and Law-C survivors had <50% injury and could reproduce. Three populations, one each for susceptible, moderately tolerant, and tolerant were sprayed with a range of glufosinate doses from 0.125 to 2x to determine the tolerance levels. The dose response assay confirmed that Pra-C and Lee-A were the least and most sensitive populations, with LD_{50} values of 344 and 141 g ha^{-1}, respectively. The basal glutamine synthetase (GS) enzyme activity of the tolerant population was 20% higher than that of the susceptible population. Ammonia accumulation was significantly higher and occurred faster in Lee-A than in Pra-C. Fifty percent of NH_3 accumulation occurred within 4 and 8 HAT in Lee-A and Pra-C, respectively. Tolerance to glufosinate is due to higher baseline activity of GS in the tolerant plants, which would require more herbicide molecule to cause substantial inhibition. However, it also seemed that GS from the tolerant plants is less sensitive to glufosinate than GS from the susceptible plants.

Nomenclature: Dicamba; glufosinate; glyphosate; Palmer amaranth, *Amaranthus palmeri*.

Key words: Differential tolerance, glutamine synthetase inhibition, tolerance mechanism.

Introduction

Glyphosate-resistant (GR) Palmer amaranth occurs in most states in the southern US. In Arkansas, GR Palmer amaranth was confirmed in 2006 (Norsworthy et al. 2008). The occurrence of GR Palmer amaranth in crop fields resulted in a concerted effort from all agriculture-related sectors to develop long-term resistance management strategies. Glufosinate is a non-selective postemergence herbicide that is being integrated into crop management systems as a tool for managing GR weeds. The LibertyLink® trait is available in soybean [*Glycine max* (L.) Merr.], canola (*Brassica napus* L.), cotton (*Gossypium hirsutum* L.), and corn (*Zea mays* L.) (Cox 1996). The glufosinate-resistance trait is conferred into crops through incorporation of the phosphinothricin-acetyl transferase (PAT) or the basta N-acetyltransferase (BAR) genes, which code for an enzyme that detoxifies glufosinate by acetylation (Bertges et al. 1994; Mullner et al. 1993). In susceptible plants, glufosinate irreversibly inhibits glutamine synthetase (GS) (Wendler et al. 1990; Wild and Mandersheid 1984; Wild and Wendler 1991), which has a pivotal role in cellular nitrogen metabolism by catalyzing the hydrolysis of glutamate and ammonia to form glutamine in the presence of the cofactors adenosine diphosphate (ADP) and inorganic phosphate (Pi) (Nkoa et al. 2003). Following GS inhibition, ammonia (NH_3) accumulates to phytotoxic levels in cells of treated plant tissues (Krieg et al. 1990). Previous studies suggested that accumulation of NH_3 results in death of the plant (Tachibana et al. 1986; Wild and Manderscheid 1984). However, a combination of several factors including inhibition of glutamine amino acid synthesis, inhibition of photosynthesis (resulting from lack of intermediates due to photorespiration interruption), and NH_3 accumulation have been attributed to plant death (Lacuesta et al. 1992; Sauer et al. 1987).

Although individual plants are visually indistinguishable, genetic differences exist. This is especially true in populations with a wide genetic diversity, like Palmer amaranth. These minor intrapopulation genetic variations confer the inherent differential ability of some plants to tolerate herbicides (Ozair 2008). The frequency of these relatively tolerant plants in a population, no matter how rare, is a primary determinant of the risk of resistance evolution (Ozair 2008; Tharayil-Santhakumar 2003).

Differential tolerance of weed species to glufosinate exists and has been attributed to application rate (Bond et al. 2006), plant species (Steckel et al. 1997), age of the plant at herbicide application (Sellers et al. 2004), time of day of application (Martinson et al. 2005), humidity, temperature (Anderson et al. 1993; Coetzer et al. 2001; Kumaratilake and Preston 2005; Petersen and Hurle 2001), and absorption and translocation (Mersey et al. 1990). Ridley and McNally (1985) observed that the differential tolerance of weed species to glufosinate was not due to differences in the degree of GS inhibition, but was mostly because of differences in absorption, translocation, and metabolism of the herbicide. Contrary to assertions by Ridley and MacNally (1985), metabolism has not been associated with differential response of weed species to glufosinate in other studies (Haas and Muller 1987; Jansen et al. 2000; Neto et al. 2000), implying that the mechanism of tolerance is species-specific. However, the fatty acid metabolites traced in plants expressing low acetylation activity (Droge-Laser et al. 1994; Muller et al. 2001; Ruhland et al. 2004) suggest that metabolism of glufosinate still occurs. Currently, glufosinate-resistant Palmer amaranth has not been documented. Resistance to glufosinate has been reported in goosegrass [*Eleusine indica* (L.) Gaertn.] (Chuah et al. 2010) and in Italian ryegrass (*Lolium perenne* L. ssp. multiflorum) (Avila-Garcia and Mallory-Smith 2011) in sites where glufosinate has been intensively used to desiccate vegetation. The evolution of GR Palmer amaranth and

increased adoption of glufosinate-tolerant crops will increase the use of glufosinate-based weed control programs, thereby increasing selection pressure on weeds.

This study aimed to (1) evaluate the response of Palmer amaranth populations to glufosinate; (2) determine the frequency of glufosinate tolerance in various populations; and (3) determine the mechanism of tolerance to glufosinate in the test populations. Findings from this research are geared to assist in understanding the response behavior of different Palmer amaranth populations to glufosinate for purposes of developing effective and specific control measures that can ameliorate management of resistance weeds.

Materials and Methods

Herbicide Tolerance Screening Assay. Greenhouse experiments were conducted in October 2010 and July 2011 at the Altheimer greenhouses, Fayetteville, Arkansas, to evaluate the response of Palmer amaranth to glufosinate. The populations used in this experiment were collected from eight counties in Arkansas: Mississippi, Lee, Lawrence, Phillips, Conway, St Francis, Prairie, and Poinsett. Palmer amaranth seeds were collected from 10 to 20 plants that had survived two glyphosate applications at a rate of 0.84 kg ae ha^{-1} in the 2009 growing season. Five hundred mg of seed from each plant were thoroughly mixed to make a composite that was used in the greenhouse experiments. Palmer amaranth seeds from the composite were planted in cellular trays (Redwayfeed Garden and Pet supply, 290 Briceland Rd, Reedway, CA 95560) with 50 cells using Sunshine premix soil (Sunshine premix #1®, Sun Gro Horticulture, 15831 NE 8th Street, Suite 100, Bellevue, WA 98008). The planted trays were placed in the greenhouse with 30/26(±4) ^{o}C day/night temperatures and watered when needed to facilitate germination and growth of the seedlings. At two-leaf stage, the seedlings were thinned to allow growth of only

one plant per cell. When Palmer amaranth seedlings were about 7-cm tall, glufosinate (Ignite 280 SL®, 280 g ai L^{-1}, Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA) was sprayed using a motorized boom with two nozzles (TeeJet® 800067 flat-fan spray nozzles, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189) spaced 51 cm apart. The doses that were evaluated in the primary screening experiments were 0.25x, 0.5x, and 1x (0.73 kg ai ha^{-1}) of glufosinate. Ammonium sulfate (AMS) was added at 20 g L^{-1}. The treatments were sprayed in 187 L ha^{-1} water at 262 kPa. The treated seedlings were, thereafter, returned to the greenhouse where they were supplied with adequate nutrients weekly and watered regularly for their survival for a 21d-period. For purposes of this study, Palmer amaranth populations were categorized as susceptible, moderately tolerant, or tolerant to glufosinate when mortality was >90%, 75 to 90%, or <75%, respectively, at the 1x rate.

Herbicide-Dose Response Assay. Based on visual ratings recorded at 21 DAT, one susceptible (S), two moderately tolerant (MT), and two highly tolerant (HT) populations were selected for further evaluation of their response to glufosinate. Palmer amaranth seeds for each population were planted in five 3-inch pots for each herbicide rate. The plants were thinned and maintained just like in the screening study. The susceptible seedlings, 7 cm tall, were treated with glufosinate ranging from 91 to 1095 g ai ha^{-1} (equivalent to 0.125 to 1.5x the recommended dose). Populations that showed some tolerance to glufosinate in the screening experiment were sprayed with 0.25 to 2x the recommended dose. The herbicide application and evaluation procedures were as in the screening experiment. In addition to visual rating and plant biomass of the survivors was recorded at 28 DAT. The experiment had three replicates and was conducted twice.

Tolerance Mechanism Studies. ***Glutamine Synthetase Enzyme Activity Assay.*** Based on the glufosinate dose required to kill 50% of the plants of each test population (LD_{50}), the most susceptible and most tolerant populations were used in the GS activity assay. The glutamyl hydroxamate synthesized from glutamate and hydroxylamine was quantified following the protocol described by Manderscheid (1993). Fresh leaf tissue (about 350 mg) was ground in liquid nitrogen and homogenized in 1.2 ml extraction buffer (pH 7.8) containing 50 mM Tris-HCl, 10 mM $MgCl_2 \cdot H_2O$, 10 mM β-mercaptoethanol, and 5% (w/v) polyvinylpyrrolidone. The crude extract was centrifuged at 16,000 *g* for 20 min at 4 ^oC. An aliquot of 200 µl supernatant was mixed with 800 µl reaction solution containing 50 mM Tris-HCl (pH 7.2), 6 mM ATP, 50 mM $MgSO_4 \cdot 7\ H_2O$, 20 mM hydroxylamine and 3.3 mM L-cysteine. The GS reaction was started by adding 150 µl 500 mM Na-glutamate and incubating at 37 ^oC for 30 min. Thereafter, 350 µl ferric chloride reagent [10% (w/v) ferric chloride in 0.2 M HCl] was added to stop the reaction. The mixture was centrifuged at 3,000 *g* for 10 min and the absorbance at 540 nm was recorded. The concentration (µM) of glutamyl hydroxamate formed per gram of fresh leaf weight was estimated using L-glutamic acid γ-monohydroxamate standard calibration curve, covering a range of 0 to 1600 µM glufosinate at 200 µM intervals.

Ammonia Accumulation Assay. The populations used in the GS activity assay were used in this experiment. Palmer amaranth seedlings (15-cm tall) were sprayed with 0.73 kg ai ha^{-1} of glufosinate. Five plants per population were assayed for NH_3 concentration at 0, 3, 6, 12, 24, 48, and 72 h after treatment (HAT). Leaves were harvested, washed with deionized water (diH_2O), and ground in liquid nitrogen. The sample (250 mg) was homogenized in 1 ml of diH_2O containing 50 mg of polyvinylpyrrolidone before centrifuging at 16,000 *g* for 7 min. The supernatant (300 µl) was diluted with 700 µl diH_2O and 100 µl of the diluted extract was mixed

with 1.5 ml of phenol nitroprusside solution before adding 1.5 ml alkaline hydrochlorite solution (2.5 g sodium hydroxide, 1.6 ml sodium hypochloride 5% available chlorine, and 500 ml diH_2O). The sample was thereafter incubated at 37 ^oC for 20 min prior to recording absorbance at 625 nm. Ammonia concentration (μg g^{-1} fresh leaf weight) was calculated from a standard calibration curve of 0 to 4.0 μg concentration using ammonium chloride (3.82 g NH_4Cl =1g NH_4^+–N) (D'Halluin et al. 1992).

Experimental Design and Data Analysis: For the screening assay, the treatments were laid out in a split-plot design with herbicide dose as the whole plot and populations as subplots. The herbicide dose and population combination were replicated four times and the experiment was conducted twice. In each replicate, 25 plants per population were evaluated. The plants were visually scored for injury 21 d after treatment (DAT) on a scale of 0 = no control to 100% = complete control. The injury and mortality data were subjected to ANOVA using general linear model procedure in SAS (SAS®, v9.2, Statistical Analysis Systems Institute, SAS Circle, P.O. Box 8000, Cary, NC 25712-8000). The run effect was insignificant and therefore the data for the two runs was pooled and analyzed together.Frequency analysis was also done to evaluate tolerance distribution of individual plants of the test populations to the 1x glufosinate dose. Fisher's Protected LSD was used to separate significant means for dose, population, and interaction effects at P = 0.05.

For the dose response experiment, the LD_{50} for each test population was determined using Sigmaplot v12 (Systat Software, Inc. 1735 Technology Drive, Suite 430 San Jose, CA 95110 USA). The amount of glufosinate that would kill 50% of the treated plants was estimated using the 3-parameter sigmoidal logistic non-linear regression at 95% confidence interval (CI).

For the GS activity assay, treatments were arranged in a split-plot design with herbicide dose as main plot and population as subplots. The experiment was replicated three times and was conducted twice. Five plants were analyzed for glutamyl hydroxamate formation per population per sampling time. The NH_3 accumulation assay was set in a randomized complete block design (blocked by day) with five plants per population, three replicates, and was conducted twice. Two-way ANOVA was performed on the log-transformed data (Coetzer and Al-Khatib 2001) in SAS to account for differences between populations, sampling times, and across repetitions. However, the run effect was insignificant and therefore the data for the two runs was pooled and analyzed together. This data analysis procedure was successfully used by Coetzer and Al-Khatib (2001).The treatment means were compared using paired t-Test and Fisher's Protected LSD at P = 0.05 was used to separate means where necessary. SigmaPlot was used to generate I_{50} (the amount of herbicide or time required for 50% inhibition of GS enzyme activity) values for GS activity assay using 3-parameter sigmoidal logistic curve (CI = 0.95).

Results and Discussion

Herbicide-Tolerance Screening Assay. A significant interaction was observed between glufosinate dose and population ($P<0.0001$). Differential response of Palmer amaranth populations to glufosinate was more common at the lower dose, but the populations responded similarly to glufosinate as the dose increased (Table 1). Glufosinate applied at 0.25, 0.5, and 1x injured Palmer amaranth populations 13 to 67, 49 to 95, and 94 to 100%, respectively, 21 DAT. However, the mortality counts differed among populations at all doses. The 0.25x dose did not kill any of the Pra-C, Mis-C, or Law-B populations. Glufosinate applied at the 1x dose killed 71 to 100% of Palmer amaranth plants, with Pra-C and Lee-A populations showing the least and

most mortality, respectively. Although, 29% of Pra-C treated plants survived the full dose, only 13% of the treated plants had <75% injury (Figure 1). The likelihood of these plants producing seeds was nil. However, 2% of Pra-C, 1% of STF-C, and 1% of Law-C survivors had <50% injury and were most likely to produce seed. These survivors can replenish the seedbank. Further, knowing that plants growing in the field are hardier than those growing in the greenhouse, it is expected that survivors from field application would be more than those from greenhouse application, or glufosinate efficacy on these plants would be less in the field as observed by Koger et al. (2005). Plants in the greenhouse grow fast and reach the recommended spraying heights earlier than in the field due to less variation in ambient carbon dioxide and temperatures. Indoor plants are also not hardened by mechanical stresses from strong winds or insect damage.

Herbicide-Dose Response Assay. The dose response assay confirmed that Pra-C tolerated glufosinate and Lee-A was susceptible, with LD_{50} values being 141 and 344 g ha^{-1} glufosinate, respectively (Figure 2). However, Law-C, which was categorized as moderately tolerant in the screening study, was similar to the susceptible in the dose response study, even though it had a higher LD_{50} *per se* than the susceptible Lee-A. This suggests that the variability at the higher glufosinate dose among individual plants within the populations was higher in Law-C than in Lee-A. The difference observed on response of Law-C reflects the degree of variability in response to herbicides among plants, especially with Palmer amaranth due to its wide genetic diversity. Herbicide response can vary among biotypes of a weed species (Patzoldt et al. 2002).

Glutamine Synthetase Enzyme Activity. GS has a pivotal role in cellular nitrogen metabolism by catalyzing the biosynthetic ATP-dependent condensation of ammonia with glutamate to form glutamine. In this study, the decline in GS enzyme activity highly correlated with the increase in

glufosinate dose, with R^2 values of 0.96 and 0.91 for Lee-A and Pra-C populations, respectively (Figure 3). Glufosinate doses of up to 600 µM inhibited GS enzyme activity linearly. Significant GS inhibition occurred up to 1000 µM ($P<0.0001$) in both populations, with no further significant reduction in GS activity above 1000 µM herbicide concentration. With ryegrass, the GS enzyme inhibition by glufosinate was linear between 0 and 400 µM and it was suggested that resistance was conferred by an altered target site (Avila-Garcia and Mallory-Smith 2011; Avila-Garcia et al. 2012). The I_{50} doses for Lee-A and Pra-C populations were 645.6 ± 71.9 and 1152.4 ± 147.9 µM of glufosinate. The I_{50} doses for the two populations significantly differed ($P <0.05$), implying that the tolerant population was less sensitive to glufosinate than the susceptible one. Pra-C had higher basal GS enzyme activity than Lee-A. The doses used in this experiment reduced GS activity for Pra-C and Lee-A populations by 51 and 65%, respectively. The tolerance of Pra-C to glufosinate emanates from a higher baseline GS activity than in Lee-A.

Ammonia Accumulation. NH_3 is used as a chemical marker for GS inhibition by glufosinate. NH_3 content in plant leaves increased exponentially with time after glufosinate treatment (Figure 4). The accumulation of NH_3 in Lee-A was much higher and occurred at a faster rate than in Pra-C, reflecting a rapid depletion of functional GS in the S plants due to lower levels of GS compared to T plants. Fifty percent of NH_3 accumulation in leaves of Lee-A and Pra-C occurred within 4 and 8 HAT, respectively. Within 24 h, ammonia accumulation in Lee-A leveled off whereas Pra-C continued to accumulate NH_3 gradually. At 10 and 18 HAT, NH_3 concentration in leaves had increased by 80% in Lee-A and Pra-C, respectively. Coetzer et al. (2001) reported that 80% inhibition (as indicated by ammonia accumulation) in Palmer amaranth occurred between 6 and 24 HAT. Similar times were also reported in alfalfa (*Medicago sativa* L.) (Lacuesta et al. 1989, 1993). The drastic NH_3 accumulation within 12 HAT in the susceptible

population being reported in this study was also observed in Palmer amaranth and green foxtail (Coetzer et al. 2001; Mersey et al. 1990). The NH_3 concentrations recorded in this experiment are lower than those reported in ryegrass, vetiver (*Vetiveria zizanoides* Nash.), and rice (Avila-Garcia and Mallory-Smith 2011; Chin-Ju et al. 2006; Prasertsongskun et al. 2002).This is expected because Palmer amaranth is a C4 plant, has lower photorespiration rate, and hence naturally produces less NH_3 than ryegrass and rice, which are C3 plants (Wendrel et al. 1990).

Results from this study demonstrated that differential response of Palmer amaranth populations to glufosinate exists. The frequency of tolerant individuals is relatively low, but is a strong indication that a small percentage of GR Palmer amaranth can survive the labeled rate of glufosinate. Additional control measures are necessary to control the survivors with <50% injury. The GS activity plays a role in the degree of tolerance of Palmer amaranth populations that were used in this experiment. It was evident that the tolerant population had higher basal GS activity and lower basal NH_3 concentration in the leaves. Tolerance to glufosinate is certainly due to higher baseline activity of GS in the tolerant plants, which would require more herbicide molecule to cause substantial inhibition. However, it also seemed that GS from the tolerant plants is less sensitive to glufosinate than GS from the susceptible plants. Additional experiments are needed to support this hypothesis.

Literature Cited

Anderson, D. M., C. J. Swanton, J. C. Hall, and B. G. Mersey. 1993. The influence of soil moisture, simulated rainfall and time of application on the efficacy of glufosinate-ammonium. Weed Res. 33:149-160.

Avila-Garcia, W. V. and C. Mallory-Smith. 2011. Glyphosate-resistant Italian ryegrass (*Lolium perenne*) populations also exhibit resistance to glufosinate. Weed Sci. 59:305-309.

Avila-Garcia, W. V., E. Sanchez-Olguin, A. G. Hulting, and C. Mallory-Smith. 2012. Target-site mutation associated with glufosinate resistance in Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*). Pest Manag. Sci. 38:1248-1254.

Bertges, W. J., D. A. Kinney, and E. P. Pieters. 1994. Glufosinate ammonium: review and update. North Cent. Weed Sci. Proc. Soc. 49:57.

Bond, J. A., L. R. Oliver, and D. O. Stephenson. 2006. Response of Palmer amaranth (*Amaranthus palmeri*) accessions to glyphosate, fomesafen, and pyrithiobac. Weed Technol. 20:885-892.

Chuah, T. S., L. V. Lun, C. T. San, and I. B. Sahid. 2010. Initial report of glufosinate and paraquat multiple resistance that evolved in a biotype of goosegrass (*Eleusine indica*) in Malaysia. Weed Biol. Manag. 10:229-233.

Coetzer, E., K. Al-Khatib, and D. E. Peterson. 2002. Glufosinate efficacy on Amaranthus species in glufosinate-resistant soybean (*Glycine max*). Weed Technol. 16:326-331.

Coetzer, E., K. Al-Khatib, and T. M. Laughin. 2001. Glufosinate efficacy, absorption, and translocation in amaranth as affected by relative humidity and temperature. Weed Sci. 49:8-13.

Cox, C. 1996. Herbicide factsheet: Glufosinate. J. Pest. Reform. 16:15-19.

D'Halluin, K., M. DeBlock, J. Janssens, J. Leemans, A. Reynaerts, and J. Botterman. 1992. The bar gene as a selectable marker in plant engineering. Method. Enzymol. 216:415-441.

Dröge-Laser, W., U. Seimeling, A. Puhler, and I. Bröer. 1994. The metabolites of the herbicide L-phosphinothricin (glufosinate): Identification, stability, and mobility in transgenic, herbicide-resistant, and untransformed plants. Plant Physiol. 105:159-166.

Foes, M. J., L. Liu, P. J. Tranel, L. M. Wax, and E. W. Stoller. 1998. A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci. 31:514-520.

Haas, P. and F. Muller. 1987. Behaviour of glufosinate-ammonium in weeds. Pages 1075-1082 in Proceedings of the British Crop Protection Conference-Weeds. Brighton, UK.

Jansen, C., I. Schuphan, and B. Schmidt. 2000. Glufosinate metabolism in excised shoots and leaves of twenty plant species. Weed Sci. 48:319-326.

Koger, C. H., A. J. Price, and K. N. Reddy. 2005. Weed control and cotton response to combinations of glyphosate and trifloxysulfuron. Weed Technol. 19:113-121.

Krieg, L. C., M. A. Walker, T. Senaratna, and B. D. McKersie. 1990. Growth, ammonia accumulation and glutamine synthetase activity in alfalfa (*Medicago sativa* L.) shoots and cell cultures treated with phosphinothricin. Plant Cell Rep. 9:80-83.

Kumaratilake, A. R. and C. Preston. 2005. Low temperature reduces glufosinate activity and translocation in wild radish (*Raphanus raphanistrum*). Weed Sci. 53:10-16.

Lacuesta, M., A. Munoz-Rueda, C. Gonzalez-Murua, and M. N. Sivak. 1992. Effect of phosphinothricin (glufosinate) on photosynthesis and chlorophyll fluorescence emission by barley leaves illuminated under photorespiratory and non-photorespiratory conditions. J. Exp. Bot. 43:159-165.

Lacuesta, M., B. Gonzalez-Moro, C. Gonzalez-Murua, and A. Munoz-Rueda. 1993. Time course of the phosphinothricin effect on gas exchange and nitrate reduction in *Medicago sativa*. Physiol. Plant. 89:847-853.

Manderscheid, R. 1993. Irreversible inhibition of glutamine synthetase from higher plants by the herbicide phosphinothricin. Pages 103-107 in P. Böger and G. Sandmann, eds. Target Assays for Modern Herbicides and Related Phytotoxic Compounds. Boca Raton: Lewis Publishers.

Martinson, K. B., B. R. Durgan, J. L. Gunsolus, and R. B. Sothern. 2005. Time of day of application effect on glyphosate and glufosinate efficacy. Crop Management doi:10.1094/CM-2005-0718-02-RS.

Mersey, B. G., J. C. Hall, D. M. Anderson, and C. J. Swanton. 1990. Factors affecting the herbicidal activity of glufosinate ammonium: Absorption, translocation, and metabolism in barley and green foxtail. Pestic. Biochem. Physiol. 37:90-98.

Muller, B. P., A. Zumdick, I. Schuphan, and B. Schidt. 2001. Metabolism of the herbicide glufosinate-ammonium in plant cell cultures of transgenic (rhizomania-resistant) and non-transgenic sugarbeet (*Beta vulgaris*), carrot (*Daucus carota*), purple foxglove (*Digitalis purpurea*) and thorn apple (*Datura stramonium*). Pest Manag. Sci. 57:46-56.

Mullner, H., P. Eckes, and G. Donn. 1993. Engineering crop resistance to the naturally occurring glutamine synthetase inhibitor phosphinothricin. Pages 38-47 in S.O. Duke, J. J. Menn, J. R. Plimmer, Eds. Pest Control with Enhanced Environmental Safety. Washington D.C., American Chemical Society.

Neto, F. S., H. D. Coble, and F. T. Corbin. 2000. Absorption, translocation, and metabolism of 14C-glufosinate in *Xanthium strumarium*, *Commelina diffusa*, and *Ipomoea purpurea*. Weed Sci. 48:171-175.

Nkoa, R., Y. Desjardins , and N. Tremblay. 2003. Glutamine synthetase in broccoli (*Brassica oleracea*) is regulated in vivo by reciprocal cooperativity. New Phytol. 158:139-149

Norsworthy, J. K., G. M. Griffith, R. C. Scott, K. L. Smith, and L. R. Oliver. 2008. Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) in Arkansas. Weed Technol. 22:108-113.

Ozair, C.A. 2008. Herbicide-Resistance and Weed-Resistance Management. On line book "Herbicide-Resistance and Weed-Resistance Management". Weed Science Society of America. http://www.weedscience.org/paper/Book_Chapter_I.pdf. Accessed April 14, 2012.

Patzoldt, W. L., P. J. Tranel, and A. G. Hager. 2002. Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. Crop Prot. 21:707-712.

Petersen, J and K. Hurle. 2001. Influence of climatic conditions and plant physiology on glufosinate-ammonium efficacy. Weed Res. 41:31-39.

Pornprom, T., J. Chompoo, and B. Grace. 2003. Glufosinate tolerance in hybrid corn varieties based on decreasing ammonia accumulation. Weed Biol. Manag. 3:41-45.

Pornprom, T., S. Surawattananon, and P. Srinives. 2000. Ammonia accumulation as an index of glufosinate-tolerant soybean cell lines. Pestic. Biochem. Physiol. 68:102-106.

Prasertsongskun, S., N. Sangduen, S. Suwanwong, V. Santisopasri, and H. Matsumoto. 2002. Increased activity and reduced sensitivity of glutamine synthetase in glufosinate-resistant vetiver (*Vetiveria zizanoides* Nash) cells. Weed Biol. Manag. 2:171-176.

Ridley, S. M. and S. F. McNally. 1985. Effects of phosphinothricin on the isozymes of glutamine synthetase isolated from plant species which exhibit varying degrees of susceptibility to the herbicide. Plant Sci. 39:31-36

Ruhland, M., G. Engelhardt, and K. Pawlizki. 2004. Distribution and metabolism of D/L-, L- and D-glufosinate in transgenic, glufosinate-tolerant crops of maize (*Zea mays* L ssp *mays*) and oilseed rape (*Brassica napus* L var *napus*). Pest Manag. Sci. 60:691-696.

Sauer, H., A. Wild, and W. Ruhle. 1987. The effect of phosphinothricin (glufosinate) on photosynthesis II. The causes of inhibition of photosynthesis. Z. Naturforsch. 42:270-278.

Steckel, G.J., S.E. Hart, and L.M. Wax. 1997. Absorption and translocation of glufosinate on four weed species. Weed Sci. 45:378-381.

Steckel, L. E. 2007. The dioecious *Amaranthus* spp.: here to stay. Weed Technol. 21:567-570.

Tachibana, K., T. Watanabe, Y. Sekizawa and T. Takematsu. 1986. Accumulation of ammonia in plants treated with bialaphos. J. Pestic. Sci. 11:33-37.

Tharayil-Santhakumar, N. 2003. Mechanism of Herbicide Resistance in Weeds. On line book "Mechanism of Herbicides Resistance in Weeds". Weed Science Society of America. http://www.weedscience.org/paper/Mechanism%20of%20Herbicide%20resistance.PDF. Accessed April 17, 2012.

Tsai, C. J., C. S. Wang, C. Y. Wang. 2006. Physiological characteristics of glufosinate resistance in rice. Weed Sci. 54:634-640.

Wendler, C., M. Barniski, and A. Wild. 1990. Effect of phosphinothricin (glufosinate) on photosynthesis and photorespiration of C3 and C4 plants. Photsynth. Res. 24:55-61.

Wild, A. and R. Manderscheid. 1984. The effect of phosphinothricin on the assimilation of ammonia in plants. Z. Naturforsch. 39:500-504.

Table 1. Glyphosate-resistant Palmer amaranth control with glufosinate in the greenhouse, Fayetteville, Arkansas, 2011.

	Palmer amaranth control[a]					
	Injury			Mortality		
Population	0.18[b]	0.37	0.73	0.18	0.37	0.73
	----------%----------			----------%----------		
Con-A	40	92	99	7	57	94
Cri-C	55	95	99	2	74	92
Law-B	12	88	99	0	38	93
Law-C	62	93	98	12	73	85
Lee-A	59	94	100	21	59	100
Mis-B	29	90	99	4	57	91
Mis-C	13	90	98	0	60	90
Phi-B	23	80	100	3	33	100
Pra-C	20	49	94	0	3	71
STF-A	46	87	100	15	53	100
STF-B	67	77	99	25	32	95
STF-C	52	92	97	11	52	88
[c]LSD		9			13	
[d]LSD		10			14	

[a] Injury based on visual rating 21 d after treatment on a scale of 0 to 100%; 0 = no injury, 100 = total death. Mortality calculated as (dead plants/total plants treated with glufosinate*100).

[b] Glufosinate dose in kg ai ha^{-1}.

[c] LSD to compare population means at the same rate.

[d] LSD to compare glufosinate dose means at the same or different populations.

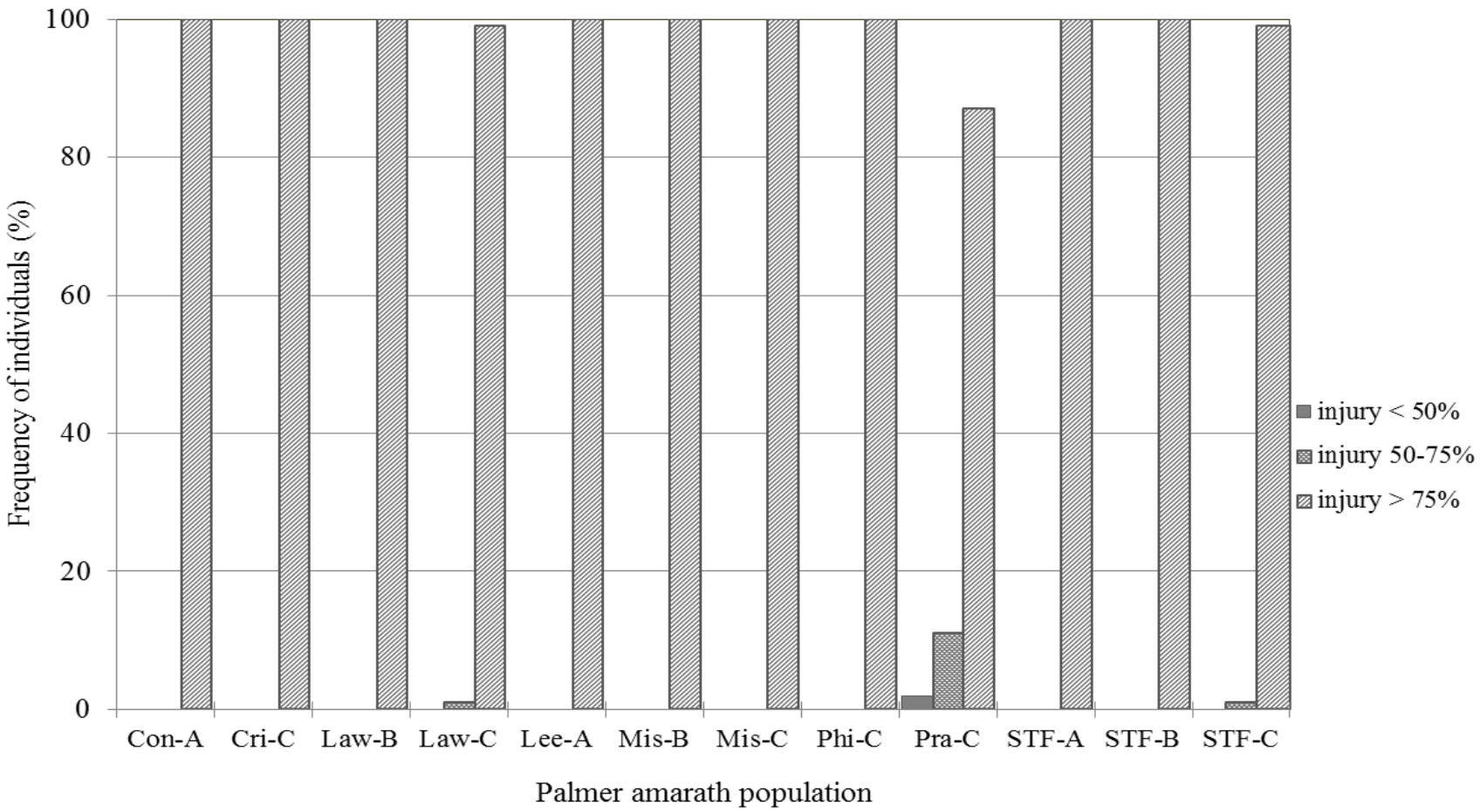

Figure 1. Frequency of plants from different Palmer amaranth populations surviving 0.73 kg ai ha^{-1} glufosinate.

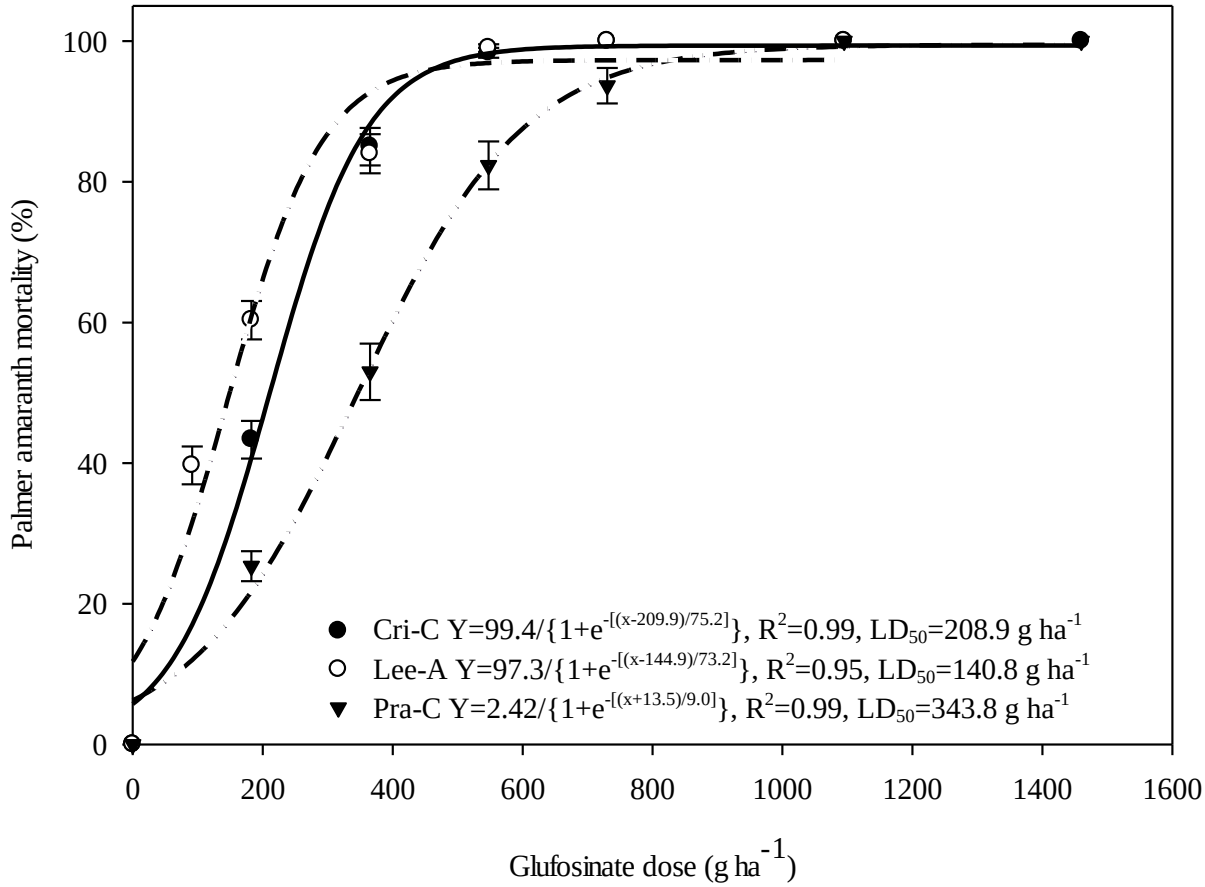

Figure 2. Response of susceptible (open circle), moderately tolerant (filled circle), and highly tolerant Palmer amaranth (filled triangle) to glufosinate in the dose range below 1600 g ha^{-1}. Vertical bars represent the SE of the mean from 15 samples.

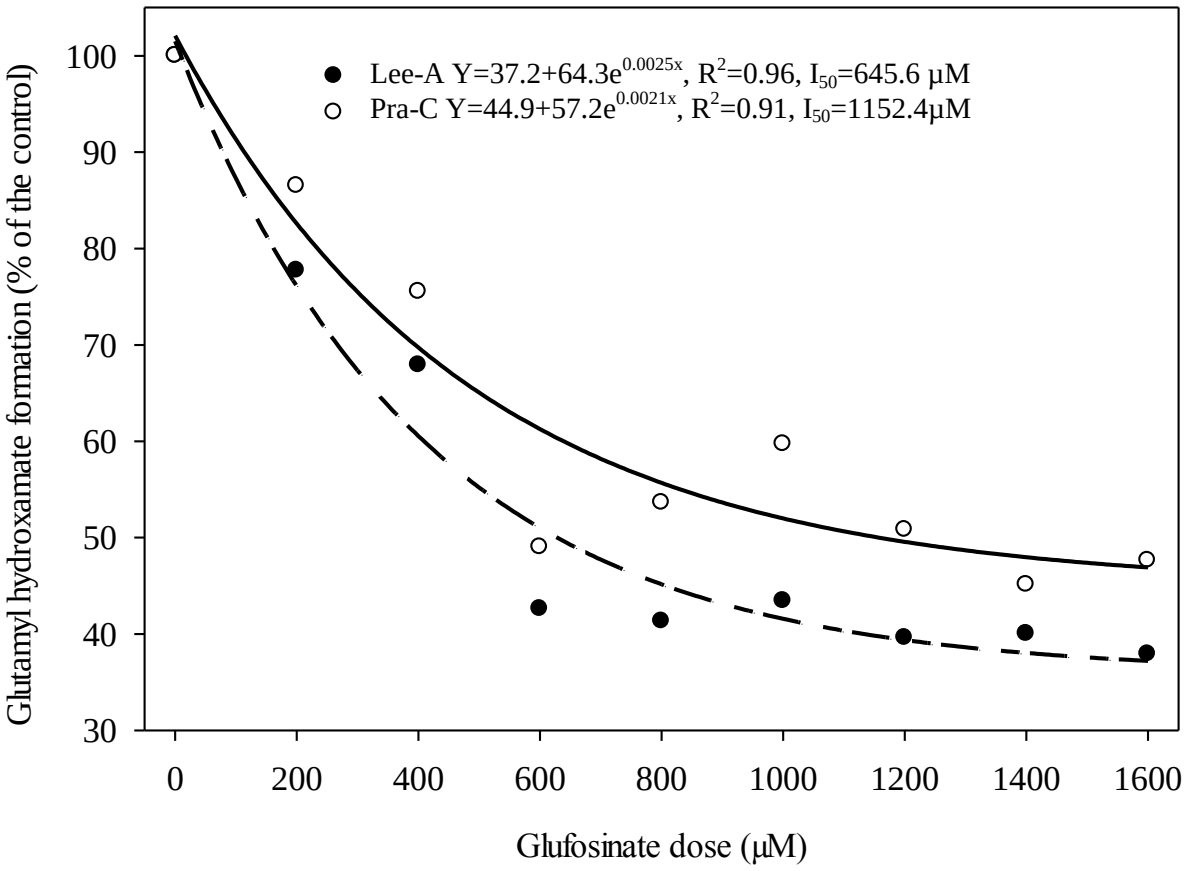

Figure 3. Formation of glutamyl hydroxamate as influenced by glufosinate dose in Lee-A (filled circle) and Pra-C (open circle) populations, expressed as % of the nontreated. Each data point is the average of 15 observations.

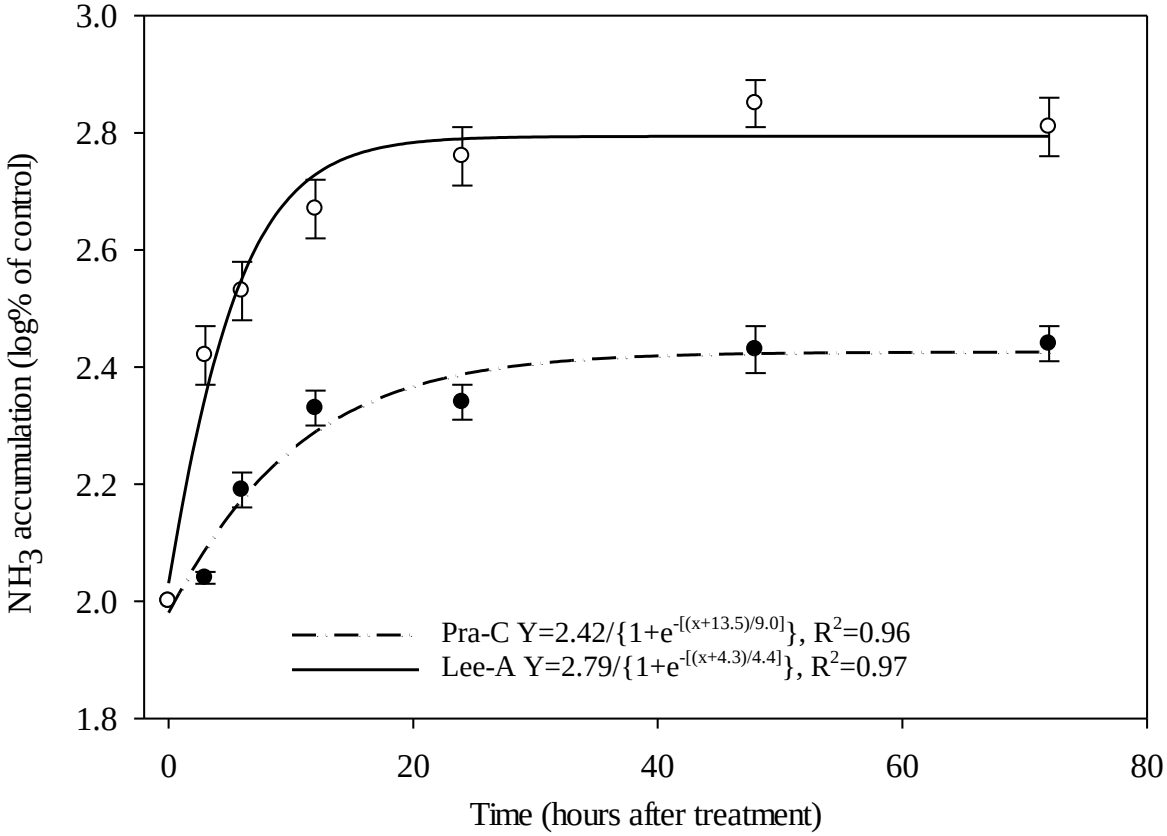

Figure 4. Ammonia accumulation in Lee-A (susceptible) (open circle) and Pra-C (tolerant) (filled circle) populations treated with 730 g ha^{-1} glufosinate. Vertical bars represent the standard errors of the mean for 30 samples. Ammonia concentration is expressed as log[(NH_3 of treated plants)/(NH_3 of nontreated)*100].

CHAPTER VI

Glyphosate-Resistant Palmer Amaranth Control in LibertyLink® Corn

Abstract

Replicated field experiments were conducted at Fayetteville and Kibler, Arkansas to investigate the response of three glyphosate-resistant Palmer amaranth populations to glufosinate, tembotrione, and 2,4-D in corn. Glufosinate and 2,4-D were applied at 0.5 and 1x their respective commercial rates of 0.73 and 1.06 kg ha^{-1}; tembotrione was applied at 1 and 2x 0.08 kg ha^{-1}. An additional blanket application of nicosulfuron and *S*-metolachlor was also made to control the grasses. The factors with the strongest effects on weed control, crop injury, and yield were location and herbicide. The additional Palmer amaranth control from blanket applications of nicosulfuron at Fayetteville was between 30 to 45%. Glufosinate applied at 1x provided the highest control at both locations. Tembotrione applied at 1x did not effectively control the test populations and up to 18% of the treated plants survived. Pra-C was the least sensitive population to all the herbicides applied at <1x rate. Although 2,4-D applied at 1x controlled Palmer amaranth at Fayetteville, it also injured 9% of the corn in the first year. No injury was observed in the second year. Corn yield was higher at Fayetteville than at Kibler because irrigation at Kibler did not meet the daily crop water requirement. No yield reduction from weeds or injury from herbicides was observed at Kibler. The study showed that good control of glyphosate-resistant Palmer amaranth can be achieved with a single-pass application of glufosinate or 2,4-D. However, the efficacy of these herbicides differed among populations and between seasons or locations.

Nomenclature: glufosinate; tembotrione, 2,4-D amine; Palmer amaranth, *Amaranthus palmeri*, corn, *Zea mays*, nicosulfuron.

Key words: Differential tolerance, corn weed control, glyphosate-resistant Palmer amaranth.

Introduction

Corn, (*Zea mays* L.*)*, known as maize across many parts of the world, is the globe's most commonly grown and widely used cereal crop. According to the United States Department of Agriculture (USDA) 2011 corn production report, 828 million metric tons of corn was produced on 163 million hectares worldwide. The United States (US) produces about 40% of the world's total production and is the world's largest corn producer. Iowa, Illinois, Minnesota, and Nebraska states account for about 50% of US corn production (USDA 2011). Corn is a staple food in most developing countries; most industrialized countries use it for animal feed or as food and feed ingredients (such as high fructose corn syrup, corn starch and lysine), or for industrial products such as ethanol. In the US, corn is mainly used for feed, residuals, and ethanol production (USDA 2011).

Palmer amaranth (*Amaranthus palmeri* S. Wats) is considered as the major weed problem in the southern Great Plains (Massinga et al. 2001) and is the most aggressive weedy species of the *Amaranthus* genus (Whitson et al. 2002). The problem of Palmer amaranth has increased throughout the southeastern US and some corn producing states (Kansas, Kentucky, Missouri, and Nebraska) more than other weedy *Amaranthus* species (Horak and Loughin 2000; Currie et al. 1998; Horak 1997), causing great concern among agriculture practitioners and researchers. The occurrence of populations that are resistant to herbicides that inhibit acetolactate synthase (ALS) (Gaeddert et al. 1997; Horak and Peterson 1995; Sprague et al. 1997), photosynthesis (Heap 2012), and 3-enolpyruvylshikimate 5-phosphate (EPSP) synthase (Culpepper et al. 2006; Norsworthy et al. 2008; Whitaker et al. 2007) complicates further the difficulties in controlling Palmer amaranth. The competitive ability of Palmer amaranth is due to its fast growth (Horak and Loughin 2000), high fecundity (Sellers et al. 2003), good light interception (Ehleringer

1983), and high water use efficiency. When compared with other C4 plants that exhibit similar water use efficiency, such as corn, Palmer amaranth survives water stress better (Downton 1975; Massinga et al. 2001; Wiese and Vandiver 1970). Palmer amaranth can, therefore, grow and reproduce under conditions that most crops would not thrive in. The ability to outcross with other *Amaranthus* species like *A. rudis* (Franssen et al. 2001; Tranel 2002) and season-long emergence (Jha and Norsworthy 2009; Keeley et al. 198) are other attributes that make Palmer amaranth a successful weed. It has also been reported that Palmer amaranth has allelopathic effects on selected plants such as cabbage (*Brassica oleracea* var. capitata), carrot (*Daucus carota* L.), onion (*Allium cepa* L.), and grain sorghum (*Sorghum bicolor* L.) (Menges 1987).

Palmer amaranth has been reported to reduce yields or interfere with harvesting mostly in cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L.). At densities of 0.32 and 10 plants m^{-1}, Palmer amaranth reduced cotton and soybean yields by 28 and 68%, respectively (Klingaman and Oliver 1994; Smith et al. 2000). Although corn generally competes with Palmer amaranth relatively better than other crops (Culpepper et al. 2010), dryland and irrigated corn yield loss has been reported (Liphadzi and Dille 2006; Massinga et al. 2001, 2003; Rule 2007). Massinga et al. (2001) reported that one and four Palmer amaranth plants m^{-1} of row emerging with irrigated corn reduced yield by 24 to 47% and 50 to 86%, respectively, across four site-years in southwestern Kansas. Massinga and Currie (2002) reported 11 to 91% corn yield reduction from 0.5 to 8 Palmer amaranth plants m^{-1} of row in Kansas. Rule (2007) found that one to four Palmer amaranth plants m^{-1} of row reduced irrigated corn yield by 39 and 52%; the same density of Palmer amaranth reduced dryland corn yield by 87 and 99%. Liphadzi and Dille (2006) predicted a 13% yield loss from 3 plants m^{-1} of Palmer amaranth that escaped herbicide treatment. They further reported dryland and irrigated corn yield losses of 6 to 60% and 5 to

38%, respectively, for Palmer amaranth densities of 0.25 to 6 plants m^{-1} of row. Yield loss due to Palmer amaranth has also been reported in grain sorghum (*Sorghum bicolor* (L.) Moench ssp. *bicolor*) (Moore et al. 2004; Weise and Vandiver 1970).

Because of GR Palmer amaranth occurrence, its ability to compete with crops, and evolution of resistance to some widely used herbicide, there is a need to evaluate the efficacy of alternative herbicides that can be integrated into management of glyphosate-resistant Palmer amaranth in corn. In the past, glufosinate and 2,4-D used to be applied as burndown treatments for controlling weeds. However, the development of various glufosinate-resistant cultivars now and in the future allows application of the herbicide directly on the crop. Glufosinate is not as effective as glyphosate on Palmer amaranth (Corbett et al. 2004), but can provide effective control when applied timely (Culpepper 2000; Gardener et al. 2006). Over 90% control of GR Palmer amaranth has been reported in other experiments (Doherty et al. 2009; Drake et al. 2009; Mohamed 2011). Tembotrione has been used in corn production to control Palmer amaranth, with mixed results. Bararpour et al. (2011) reported that 0.092 kg ha^{-1} tembotrione controlled Palmer amaranth 90 to 100%. Lally (2011) reported similar results, where 0.092 kg ha^{-1} tembotrione controlled 100% susceptible Palmer amaranth biotype. However, 66 to 82% of the populations suspected to be HPPD-resistant were controlled in the greenhouse 28 d after application. 2,4-D has also been used to control Palmer amaranth with variability in efficacy ranging from 66 to 90% in GR Palmer amaranth biotypes (Edwards 2011; Jha et al. 2009; Nandula et al. 2011). Studies were therefore conducted to determine the effects of glufosinate, tembotrione, and 2,4-D on Palmer amaranth control, and corn yield variability.

Materials and Methods

Experiment Site. Replicated field experiments were conducted in 2011 and 2012 at the Arkansas Agricultural Research and Extension Center (AAREC) with Captina silt loam soil (fine-silty, siliceous, active, mesic Typic Fragiudults) at Fayetteville, Arkansas, and in 2012 at the Vegetable Research Station (VRS) with Roxana silt loam soil (coarse silty, mixed, superactive, nonacid, thermic Typic Udifluvents) at Kibler, Arkansas.

Experiment Procedures. The experimental site at AAREC was tilled and bedded (91 cm interrow spacing) prior to planting corn; the plots at VRS were tilled, and corn was planted on the flat. Prior to planting, nitrogen (N), phosphorus (P), and potassium (K) were applied at 56, 112, and 56 kg ha^{-1}. This was followed by N application at 100 and 160 kg ha^{-1} at Fayetteville and Kibler, respectively, two weeks after planting. The corn varieties planted were P1184HR (Pioneer Hi-Bred International Inc., Resource Connection, PO Box 1000, Johnston, IA 50131-0184) in 2011at Fayetteville and TMF2Q716F2 (Dow AgroSciences LLC, 9330, Zionsville Road, Indianapolis, IN 46268) at both stations in 2012 at a density of 83,000 plants ha^{-1}. Pra-C, Mis-C, and STF-C Palmer amaranth populations were hand-seeded in 6-m rows 15 cm away from corn rows 5 d after corn planting in 2011 and on the same day of planting corn in 2012. Pra-C, Mis-C and STF-C had shown higher tolerance to glufosinate and tembotrione in the greenhouse studies, hence their inclusion in this test. The experimental plots were irrigated the soonest possible time after planting to ensure uniform germination.

When Palmer amaranth seedlings were about 7-cm tall, glufosinate (Ignite 280 SL®, 280 g ai L^{-1}, Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA), tembotrione (Laudis®, 419 g ai L^{-1}, Bayer CropScience, 26 Davis Drive, Research Triangle Park, NC 27709, USA), and 2,4-D amine (Weedar 64®, 455 g ae L^{-1}, Nufarm Inc, 150 Harvester Drive,

Burr Ridge, IL, 60527, USA) were applied with a CO_2 backpack sprayer calibrated to deliver 187 L ha^{-1} at 248 kPa. Glufosinate and 2,4-D were applied at 0.5 and 1x their labeled rates of 0.73 and 1.06 kg ha^{-1}, respectively. Tembotrione was applied at 1and 2x the labeled rate of 0.08 kg ai ha^{-1}. The 2x rate was included because tembotrione at 1x dose controlled only 60 to 71% of Pra-C, STF-C and Mis-C in earlier greenhouse studies. Ammonium sulfate (AMS) was added at 10 and 20 g L^{-1} to tembotrione and glufosinate tanks, respectively. Methylated seed oil was added to tembotrione treatments at 1% (v/v). Plots at AAREC were maintained free of grass weeds by POST application of 35 g ha^{-1} nicosulfuron (Accent®, DuPont, 1007 Market Street, Wilmington, DE 19898) and *S*-metolachlor (Dual Magnum ®, Syngenta, Syngenta Crop Protection, LLC Greensboro, NC 27419) a week after treatment application. Nicosulfuron was used because it has minimal activity while *S*-metolachlor has no POST activity on Palmer amaranth (Clewis et al. 2006).

Experimental Design, Data Collection, and Analysis. The treatments were established in a split-plot design at both sites with herbicide and Palmer amaranth population as the main plot and subplot, respectively. Palmer amaranth control data were collected from the middle 2-m length of the subplots. In order to determine the crop and weed injury, corn and Palmer amaranth stand count data were recorded before and 21 d after spraying the herbicides. The corn was hand-harvested from a representative 2-m length of row, and grain yield, adjusted to 13.5% moisture content (Massinga et al. 2003) was determined. Palmer amaranth control, crop injury, and grain yield data were subjected to analysis of variance using the general linear model procedure in SAS (SAS®, version 9.3, Statistical Analysis Systems Institute, SAS Circle, P.O. Box 8000, Cary, NC 25712-8000). The populations and herbicides were considered as fixed effects and location and replications (nested within location) were considered as random effects. The season

by location effect on Palmer amaranth control, crop injury, and yield across locations was not analyzed because the two locations did not have the same number of implementation years. Unlike at Fayetteville where the experiment was conducted for 2 yr, the experiment was conducted at Kibler in the second year only. The data for Fayetteville were therefore pooled and analyzed as a mean for the 2 yr, except for yield data. Fisher's least significant difference (LSD) was used to separate means where ANOVA showed differences at 5% level of significance.

Results and Discussion

Palmer Amaranth Control. A herbicide by location interaction was observed to be significant (**Error! Reference source not found.**). The difference in Palmer amaranth control at the two locations was due to additional control from nicosulfuron applied at Fayetteville in both years to control barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster]. Nicosulfuron caused 30 to 45% injury on Palmer amaranth (data not presented) in plots where the test herbicides were not applied. Although nicosulfuron is labeled mainly for grasses, it still has activity on some broadleaf weeds. Hennigh (2009) reported up to 65% control of broadleaf weeds including Palmer amaranth when nicosulfuron was mixed with rimsulfuron. Higher activity on broadleaf species from the mixture vs. nicosulfuron alone is expected. Palmer amaranth has season-long emergence. The addition of *S*-metolachlor at Fayetteville controlled late-emerging Palmer amaranth. Since we did not tank-mix nicosulfuron and S-metolachlor with the test herbicides, we could not confidently account for the control resulting from the test herbicides. Based on the herbicide activities at Kibler (which did not have any blanket application of nicosulfuron) and the Palmer amaranth injury observed in plots without the test

herbicides, the actual Palmer amaranth control from tembotrione and 2,4-D at Fayetteville might have been lower than what was recorded. Regardless of the additional control resulting from nicosulfuron and *S*-metolachlor application, differences still existed among herbicides at both locations. Glufosinate applied at 1x controlled all Palmer amaranth populations >96% at both locations. Similar control was achieved when 2,4-D was applied at 1x rate at Fayetteville (Table 2). However, Palmer amaranth control from 2,4-D was significantly lower at Kibler, especially on Mis-C population. Tembotrione at 1x controlled Palmer amaranth <54% at Kibler; its efficacy was higher at Fayetteville where at least 85% control was recorded. Tembotrione and 2,4-D applied at 1x and 0.5x rates, respectively, provided the least control on Pra-C, which was also the least sensitive population to all the herbicides applied at any rate <1x and at both stations. In earlier studies conducted in the greenhouse (not published), tembotrione controlled 79, 64, and 49% of Pra-C, STF-C, and Mis-C, respectively; on the other hand, 2,4-D controlled 96, 99, and 100% while glufosinate controlled 71, 88, and 90% of Pra-C, STF-C, and Mis-C, respectively. Palmer amaranth control at 21 DAT ranged from 23 to 100% at Kibler and from 79 to 100% at Fayetteville. The plants that survived the herbicides had flowers at the time of harvesting the crop, illustrating that they were healthy enough to produce seeds. Additional control is necessary to prevent these escapees from production.

Crop Injury and Corn Yield. Herbicide and location were the only main factors with significant effects on crop injury. Glufosinate and tembotrione applied at 1x and 2x, respectively, caused <1% injury on corn at Fayetteville in 2011; on the other hand, 2,4-D caused up to 9% injury in the same experiment (Table 2). No crop injury from all herbicides was observed at both locations in 2012. Crop injury is more likely to occur if corn is growing rapidly under high temperature, high soil moisture conditions, and is taller than 30 cm when 2,4-D is sprayed

(Anonymous 2008). However, the environmental conditions during the implementation period for the two years were similar (Table 4); therefore, the crop injury in 2011 was due to varietal differences.

The only factor with strong effect on yield was location. Yield was not affected by Palmer amaranth presence in the field (P = 0.8945). At 21 DAT, Palmer amaranth average density was 3.3 plants m^{-1} row in nicosulfuron-alone treated plots at Fayetteville. However, the plants were suppressed long enough for the corn to develop faster than the time it took for Palmer amaranth to recover from nicosulfuron injury. Unlike the results from our study, other researchers found that at 1 to 4 plants m^{-1}, Palmer amaranth that had escaped herbicide application still reduced the yield of irrigated corn by up to 13% (Liphadzi and Dille 2006; Masinga and Currie 2002; Rule 2007). The corn yield at Fayetteville was much higher than that at Kibler. The second year (2012) was extremely hot and dry. The irrigation at Kibler did not provide adequate water to meet the daily water requirement of corn at 60 d after planting and beyond

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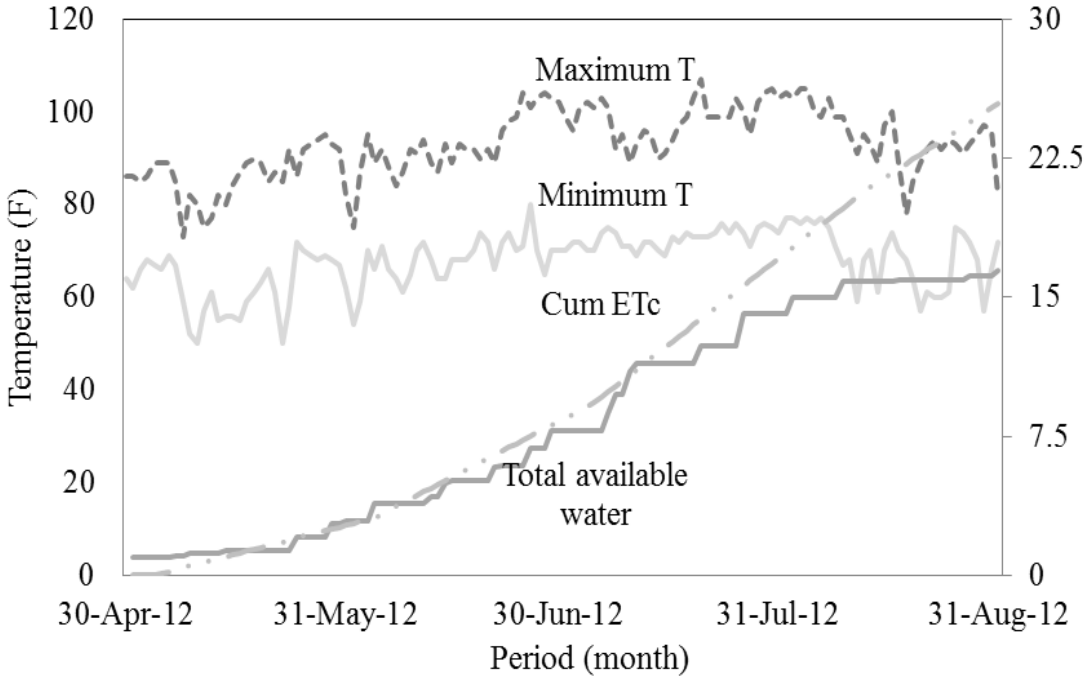

Figure 5) as evidenced by severe wilting, eventual premature desiccation of foliage, and stunted growth. This was the period when corn was silking; any moisture stress during this period can reduce yield more than stress at any other development stage. Cakir (2004) and Doorenbos and Kassam (1979) reported corn yield reduction by 52 to 93% when the crop was under water stress during silking stage. Yield differences between seasons were observed at Fayetteville, with higher yields being recorded in 2012 than in 2011. This could probably be due to the differences in the varieties that we used in the two seasons and/or delayed application of nicosulfuron in 2011, which resulted in greater competition between grass weeds and corn.

The study showed that good control of GR Palmer amaranth can be achieved with a single-pass post application of glufosinate or 2,4-D. However, it is evident that different Palmer amaranth populations show differential response between seasons or locations. As such, scouting would help to determine the control levels and plan for complementary control strategies to avoid plants escaping normal herbicide sprays from producing seeds. A season-long weed

control program needs to be planned prior to planting the crop. The full rate of tembotrione did not adequately control the test populations. This calls for integrating tembotrione with other herbicides for effective control. Tembotrione label allows for sequential application where necessary, however, frequent use of this herbicide can quickly select for tolerance in the populations evaluated in this study. Follow-up studies are needed to investigate the variability in effectiveness of tembotrione and 2,4-D at different locations. Yield and yield components were not significantly affected by herbicide or weed control levels. During harvesting, most of the surviving Palmer amaranth plants had flowers and were healthy enough to produce seeds. This illustrates the significance of controlling these survivors to ensure that they do not go into production.

Literature Cited

Anonymous 2010. 2,4-D amine label. http://www.cdms.net/LDat/ld44N012.pdf. Accessed September 16th 2012.

Clewis, S. B., J. W. Wilcut, and D. Porterfield. 2006. Weed management with *S*-metolachlor and glyphosate mixtures in glyphosate-resistant strip- and conventional tillage cotton (*Gossypium hirsutum* L.). Weed Technol. 20:232-241.

Culpepper A. S., A. C. York, S. M. Brown, W. W. Hanna, J. W. Davis, W. K. Vencill, T. L. Grey, T. M. Webster, and J. M. Kichler. 2006. Glyphosate-resistant Palmer amaranth (*Amaranthus Palmeri*) confirmed in Georgia. Weed Sci. 54:620-626.

Culpepper, A. S., T. M. Webster, L. M. Sosnoskie, and A. C. York. 2010. Glyphosate-resistant Palmer amaranth in the United States. Pages 195-212 in V. J. Nandula, ed. Glyphosate resistance in crops and weeds: History, development and management. New York: John Wiley and Sons Inc.

Currie, R., K. Kreikemeier, and R. Massinga. 1998. Impact of Palmer Pig-weed on Quality and Yield of Whole-Plant Corn. Manhattan, KS: Kansas Agricultural Experimental Station, Kansas State University Southwest Research—Extension Center Field Day Report of Progress 814. pp. 42-43.

Doherty, R. C., K. L. Smith, J. A. Bullington, and J. R. Meier. 2009. Palmer amaranth control with dicamba and glufosinate as influenced by weed size and herbicide rate. Pages 105-107 in D. M. Oosterhuis eds. Summaries of Arkansas Cotton Research. University of Arkansas, Fayetteville, AR.

Doorenbos, J. and A. K. Kassam. 1979. Yield response to water. Irrigation and Drainage Paper 33. FAO, United Nations, Rome. pp. 176.

Downton, W. J. 1975. The occurrence of C4 photosynthesis among plants. Photosynthetica 9:96-105.

Drake, D. M., L. R. Oliver, and M. T. Barapour. 2009. Palmer amaranth (*Amaranthus Palmeri*) control in soybean. Page 13 in Proceedings of the 13th research conference. Fayetteville, AR: Arkansas Crop Protection Association.

Ehleringer J. 1983. Ecophysiology of *Amaranthus Palmeri*, a sonoran desert summer annual. Oecologia 57:107-112.

Franssen, A. S., D. Z. Skinner, K. Al-Khatib, M. J. Horak, and P. A. Kulakow. 2001. Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. Weed Sci. 49:598-606.

Gaeddert, J. W., D. E. Peterson, and M. J. Horak. 1997. Control and cross-resistance of an acetolactate synthase inhibitor-resistant Palmer amaranth (*Amaranthus palmeri*) biotype. Weed Technol. 11:132-137.

Gossett, B. J., E. C. Murdock, and J. E. Toler. 1992. Resistance of amaranth (*Amaranthus palmeri*) to dinitroaniline herbicides. Weed Technol. 6:587-591.

Heap, I. M. 2012. Glycine-resistant weeds by country and species. http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go. Accessed: March 31, 2012.

Hennigh, D. S. 2009. Postemergence weed management in acetolactate synthase (ALS) resistant grain sorghum. PhD. Dissertation. Manhattan, KS. Kansas State University 18p.

Horak, M. J. 1997. The changing nature of Palmer amaranth: a case study. Proc. North Central Weed Science Society. 52:161.

Horak, M. J. and D. E. Peterson. 1995. Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. Weed Technol. 9:192-195.

Horak, M. J. and T. M. Loughin. 2000. Growth analysis of four *Amaranthus* species. Weed Sci. 48:347-355.

Jha, P. and J. K. Norsworthy. 2009. Soybean canopy and tillage effects on emergence of Palmer amaranth (*Amaranthus palmeri*) from a natural seed bank. Weed Sci. 57:644-651.

Keeley, P. E., C. H. Carter, and R. J. Thullen. 1987. Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci. 35:199-204.

Klingaman, T. E and L. R. Oliver. 1994. Palmer (*Amaranthus palmeri*) interference in soybean (*Glycine max*). Weed Sci. 42:523-527.

Lally, N. G. 2011. Pyrasulfotole and bromoxynil response in grain sorghum. MSc. Thesis. Manhattan, KS. Kansas State University 87p.

Liphadzi, K. B. and J. A. Dille. 2006. Annual weed competitiveness as affected by preemergence herbicide in corn. Weed Sci. 54:15-165.

Loux, M. M., D. Doohan, A. F. Dobbels, and W. G. Johnson. 2012. Weed control guide for Ohio and Indiana. http://agcrops.osu.edu/specialists/weeds/specialist-links/2010%20Weed%20Control%20Guide.pdf. Accessed September 16th, 2012.

Massinga, R. A. and R. S. Currie. 2002. Impact of Palmer amaranth (*Amaranthus palmeri*) on corn (*Zea mays*) grain yield and yield and quality of forage. Weed Technol. 16:532-536.

Massinga, R. A., R. S. Currie, and T. P. Trooien. 2003. Water use and light interception under Palmer amaranth (*Amaranthus palmeri*) and corn competition. Weed Sci. 51:523-531.

Massinga, R. A., R. S. Currie, M. J. Horak, and J. Boyer, Jr. 2001. Interference of Palmer amaranth (*Amaranthus palmeri*) in corn (*Zea mays*). Weed Sci. 49:202-208.

Menges, R. M. 1987. Allelopathic effects of Palmer amaranth (*Amaranthus Palmeri*) and other plant residues in soil. Weed Sci. 36:325-328.

Mohamed, A. T. A. 2011. Control, assessment and glyphosate resistance of Palmer amaranth (*Amaranthus palmeri* S. Wats) in Virginia. MSc Thesis. Blacksburg, VA. Virginia Polytechnic Institute and State University. 26p

Monks, D. W. and L. R. Oliver. 1988. Interactions between soybean (*Glycine max*) cultivars and selected weeds. Weed Sci. 36:770-774.

Moore, J. W., D. S. Murray, and B. Westerman. 2004. Palmer amaranth (*Amaranthus palmeri*) effects on harvest and yield of grain sorghum (*Sorghum bicolor*). Weed Technol. 18:23-29.

Norsworthy, J. K., G. M. Griffith, R. C. Scott, K. L. Smith, and L. R. Oliver. 2008a. Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol. 22:108-113.

Rule, D. M. 2007. Corn and Palmer amaranth interactions in dryland and irrigated environments. PhD Dissertation. Manhattan, KS. Kansas State University. 29p.

Sellers, B. A., R. J. Smeda, W. G. Johnson, J. A. Kendig, and M. R. Ellersieck. 2003. Comparative growth of six *Amaranthus* species in Missouri. Weed Sci. 51:329-333

Smith D. T., G. L. Steele, and R. V. Baker. 2000. Palmer amaranth (*Amaranthus Palmeri*) impacts on yield, harvesting, and ginning in dryland cotton (*Gossypium hirsutum*). Weed Technol. 14:122-126.

Sprague, C. L., E. W. Stoller, L. M. Wax, and M. J. Horak. 1997. Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) resistance to selected ALS-inhibiting herbicides. Weed Sci. 45:192-197.

Tranel, P. J., J. J. Wassom, M. R. Jeschke, and A. L. Rayburn. 2002. Transmission of herbicide resistance from a monoecious to a dioecious weedy *Amaranthus* species. Theor. Appl. Genet. 105:674-679.

Whitaker J., A. C. York, and A. S. Culpepper. 2007. Glyphosate-resistant Palmer amaranth distribution and control in North Carolina and Georgia. Page 1226-1227 in Proceedings of the Beltwide Cotton Conference. Memphis, TN. National Cotton Council of America.

Whitson, T. D., L. C. Burrill, S. A. Dewey, D.W. Cudney, B. E. Nelson, R. D. Lee, and R. Parker. 2002. 9th ed. Weeds of the West. Newark, CA: Western Society of Weed Science, the Western United States Land Grant Universities Cooperative Extension Services, and the University of Wyoming. pp. 2.

Wiese, A. F. and C. W. Vandiver. 1970. Soil moisture effects on competitive ability of weeds. Weed Sci. 18:518-519.

Table 1.. Effects of location, herbicide, Palmer amaranth population, and their interaction on Palmer amaranth control, crop injury and yield of corn.

Experimental factor	Effect[a]		
	AMAPA[b] control	Crop injury	Yield
Location	<0.0001	0.0143	<0.0001
Herbicide	<0.0001	0.0012	NS
Population	NS	NS	NS
Location*Herbicide	<0.0001	NS	NS
Herbicide*Population	NS	NS	NS
Location*Population	NS	NS	NS
Location*Herbicide *Population	NS	NS	NS

[a] Effects determined in SAS at 5% lever of significance. Values <0.05 show significant effect; NS stands for insignificant effect.

[b] Abbreviation: Palmer amaranth. Palmer amaranth control and crop injury data were collected 21 DAT.

Table 2. Palmer amaranth control, crop injury, and corn yield after treatment with glufosinate, 2,4-D, and tembotrione at Fayetteville and Kibler, Arkansas, for 2011 and 2012.

		Palmer amaranth control (%)[a]			Crop injury[a]	Yield (kg ha^{-1})	
Herbicide	Rate (kg ha^{-1})	Mis-C	Pra-C	STF-C	(%)	2011	2012
		----------Fayetteville----------					
Glufosinate	0.37	84.4	79.2	88.8	0.0	8456	14222
Glufosinate	0.73	100.0	96.3	99.0	0.1	9222	15451
Tembotrione	0.08	84.7	79.7	96.3	0.0	8105	13938
Tembotrione	0.16	97.6	92.3	100.0	0.6	9982	16044
2,4-D	0.53	98.5	92.3	94.9	6.3	8448	12603
2,4-D	1.06	100.0	100.0	98.8	8.5	8302	13476
		----------Kibler----------					
Glufosinate	0.37	90.2	85.1	97.7	0.0	-	2097
Glufosinate	0.73	100.0	100.0	100.0	0.0	-	3292
Tembotrione	0.08	53.6	32.7	38.3	0.0	-	2445
Tembotrione	0.16	82.7	97.4	89.0	0.0	-	2070
2,4-D	0.53	73.0	23.4	49.2	0.0	-	1325
2,4-D	1.06	67.8	91.4	89.2	0.0	-	2530
[b]$LSD_{0.05}$			17.5		3.3		1308
[c]$LSD_{0.05}$			12.9		2.8		NS
[d]$LSD_{0.05}$			NS		NS		NS

[a] Data collected 21 DAT: Palmer amaranth control and Crop injury calculated as $[(SC_1-SC_2)/SC_1]*100$ where SC_1 and SC_2 are stand counts before and 21 d after spraying, respectively. The data at Fayetteville were averaged across the years.

[b] Fisher's Protected LSD to compare location means at the same or different combination of herbicide and population; [c] LSD to compare herbicide means at the same location and accession; [d] LSD to compare accession means at the same location and herbicide combination.

Table 4. Mean values of weather data for Fayetteville and Kibler over four months (30th April to 31st August).

		Environmental factor		
Location	Year	Min T ^{o}C	Max T ^{o}C	Rainfall (mm)
Fayetteville	2011	20 ± 3	31 ± 7	371
Fayetteville	2012	18 ± 10	30 ± 9	221
Kibler	2012	18 ± 9	32 ± 5	198

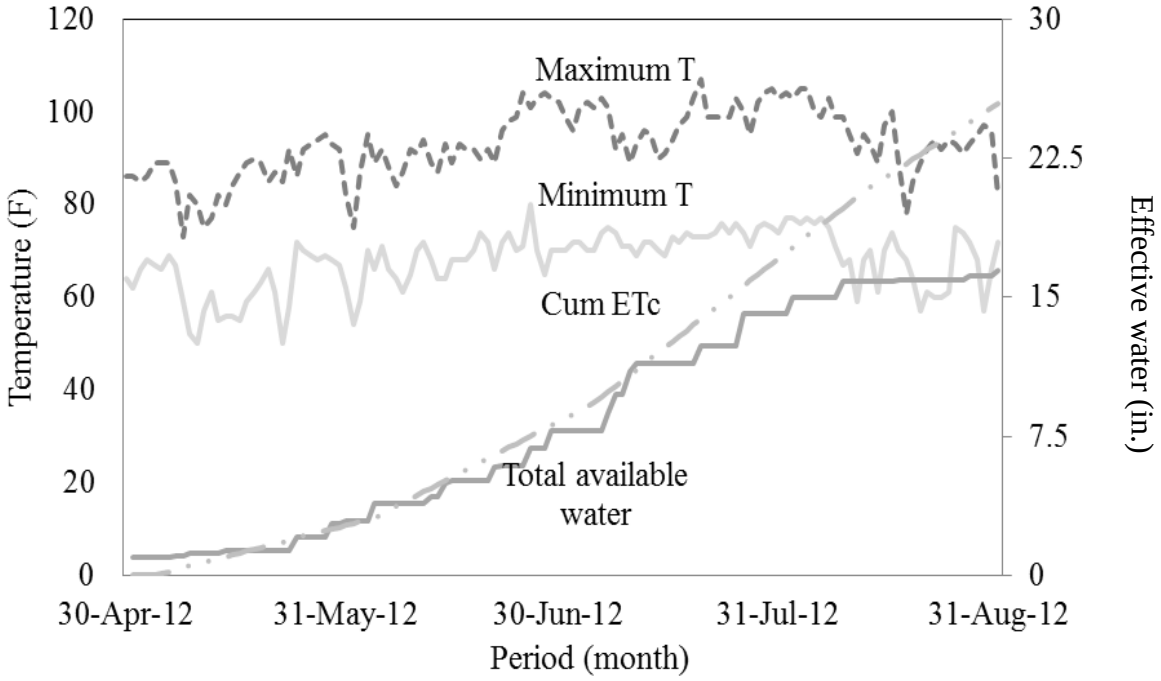

Figure 5. Daily maximum and minimum temperatures, effective water, and crop water requirement (ETc) during the experiment period, Kibler 2012. Effective water availability was calculated as precipitation + irrigation; ETc is based on the irrigation recommendations for Arkansas corn (MP437).

CONCLUSIONS

Glyphosate-resistant Palmer amaranth has become a major weed problem in upland areas in the southern US. The glyphosate-resistant Palmer amaranth populations collected from eight counties in Arkansas showed differential response to glufosinate, tembotrione, 2,4-D, and dicamba. In general, only a small percentage of glyphosate-resistant Palmer amaranth can survive the labeled rate of glufosinate, 2,4-D and dicamba. However, dicamba should be applied at 2x rate or in combination with other strategies in order to kill more of the survivors having less than 50% injury when 1x dicamba is applied. Selection for herbicide resistance to dicamba would easily occur given the above scenario. Similarly, tembotrione would need increased rates, or sequential application, or herbicide mixture to maximize control. The tembotrione label allows for sequential application where necessary. However, increased and frequent use of this herbicide can quickly select for tolerance in the populations evaluated in this study. When compared with the glufosinate, 2,4-D, and dicamba, tembotrione has the highest risk of selecting for resistant. For the populations that tolerated glufosinate, the glutamine synthetase (GS) activity plays a role in the degree of tolerance. Tolerance to glufosinate is primarily due to higher baseline activity of GS in the tolerant plants, which would require more herbicide molecule to cause substantial inhibition. When tank-mixed, the majority of glufosinate + tembotrione and some glufosinate + dicamba tank-mixtures were not compatible. Based on these findings, mixing tembotrione and glufosinate would just be costly to growers, with little or no additional benefit on Palmer amaranth control. On the other hand, 2,4-D + glufosinate tank mixtures were generally additive and in few cases, synergistic. Glufosinate and 2,4-D combinations at 0.5x and 1x rates have also shown to be able to control Palmer amaranth 100%. In corn, good control of glyphosate-resistant Palmer amaranth can be achieved with a single-pass post application of

glufosinate or 2,4-D. However, it is evident that different populations show differential response between seasons or locations. Diversified weed management strategies should therefore be integrated to maximize Palmer amaranth control. Palmer amaranth plants that survived the herbicides were outcompeted by corn, with no effect on corn yield. However, most of the surviving plants had flowers and were healthy enough to produce seeds. This illustrates the significance of controlling these survivors to ensure that they do not go into production.